NATURE

No. 4802 SATURDAY, NOVEMBER 11, 1961 Vol. 192

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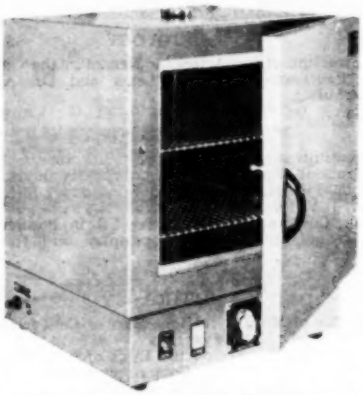

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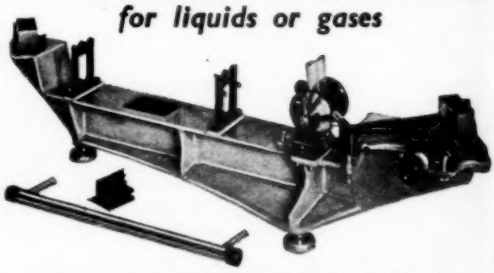

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CONTROL OF GOVERNMENT EXPENDITURE ON RESEARCH

THE Council for Scientific and Industrial Research, since its constitution some four years ago, has been concerned with the most effective means of budgetary control of scientific research in Britain. In its 1958 report the Council pointed out that, while it was possible to estimate research needs fairly accurately two years ahead, this could only be done provisionally five years in advance, and, accordingly, the Council sought as much flexibility as possible in allocating the funds at its disposal. A year later the Council stated that the Treasury had agreed that, without prejudice to the rest of the five-year plan to which the Council seeks to work, the Council might enter into commitments for research grants at a substantially higher level than was possible with the sums provided in the plan and that this level should be reviewed year by year.

In the Council's latest report, this policy was taken a stage further and the introduction was announced of an experimental scheme which should be more adaptable to rapid and unpredictable change. Under it the Council proposes each year to try to forecast its needs for five years in advance and to agree with the Treasury, subject to approval by Parliament, first the expenditure for the following year; secondly, reasonably firm estimates for the two succeeding years; and thirdly, less-definite estimates for the fourth and fifth years. This means that each year the estimates will be raised and brought forward one year, so that expenditure of each year will be planned against a fairly clear indication of probable expenditure in the years immediately following and with some regard to longer-term plans. Major departures from the estimates for the second and third years would only be made if there were important unforeseen changes in the scientific or financial situation.

This scheme seems to meet one comment made by the Select Committee on Estimates in its Fifth Report for the Session 1957–58, reviewing the work of the Department, when the Committee expressed the view that the timing of the termination of a piece of research could be as important as the timing of its initiation, and welcomed the Department's practice of regular reviews, not tied to the approval of the annual programmes, working independently of the advisory boards of the establishments. Strong support for this more flexible policy is now forthcoming from the Committee which, under the chairmanship of Lord Plowden, reported last June on the *Control of Public Expenditure**. This support is perhaps the more significant, first because although the Plowden Committee was not appointed to deal specifically with scientific or technical expenditure, it quickly distinguished the difficulties which characterize the control of such expenditure to-day; and secondly, because the difficulties encountered by the Council for Scientific and Industrial Research arise not simply from the mounting cost of scientific work but also from the effect of interlocking decisions taken elsewhere, as in educational policy, or in the fields of fuel, power, communications, space research, and the like.

Under the traditional system in Great Britain, as it developed in the nineteenth century and has continued throughout the lifetime of successive Governments to the present day, the tendency is for decisions on expenditure to be taken piecemeal. It is increasingly apparent that, where science and technology are concerned especially, this system is wasteful and liable to cause frustration and waste of resources of finance and material as well as of man-power. This is fully recognized by the Plowden Committee, and the core of its proposals is the development and use by Government of long-term surveys of expenditure and resources.

The report is, in fact, a State paper of outstanding significance which deserves—and indeed demands—close study by all who are concerned with the public finance of research and development and the most effective allocation of available resources. It is a sequel to an earlier report on Treasury Control of Expenditure from the Select Committee on Estimates in 1958, and the Committee was appointed in the summer of 1959. Several confidential reports have already been submitted to the Chancellor of the Exchequer, Mr. Selwyn Lloyd, and his predecessor, and the present report is a consolidation of those suitable for publication and discusses more especially the principles and practice of the control of public expenditure. It should stimulate exactly that constructive thinking and foresight which are imperative in dealing with many of the proposals for research and development projects to-day. In the judgment of the Committee, the crucial questions are not those of detail—the precise nature of the organization and chains of command within and between Departments, important though these are. The real problems are the wider issues of the purpose which the Government organization is attempting, its attitudes, what it regards as important, and its approach to its work on all matters involving public expenditure. Such issues are not always clear-cut, nor do they lend themselves readily to specific recommendations; but the scientist or administrator who reads this report side by side, for example, with the concluding passages of Sir Charles Snow's *Science and Government*, may find it a suggestive experience, for the whole report is designed to indicate fruitful lines of development in the future.

At the outset the Committee pays a handsome and deserved tribute to the work of the Civil Service which scientists should note. Changes, both in scope and character, in the activities of Government during the past two decades have produced strains in

* *Control of Public Expenditure*. Pp. 33. (Cmnd. 1432.) (London: H.M.S.O., 1961.) 1s. 9d. net.

traditional structures and practices, and Lord Plowden and his colleagues have been impressed by the way the Civil Service has adapted itself to deal with these changes. In particular, notable progress has been made in inter-departmental co-operation: there is a growing realization that in place of single departments dealing with individual problems in comparative isolation, the public interest requires a team pursuing its common objectives with a single purpose. This attitude of mind, and the readiness to initiate changes to improve control of expenditure, were manifested throughout the discussions between the Committee and the permanent secretaries and other officials of the major Departments. Furthermore, members of the Committee with experience of industry and commerce, and the specialist advisers who joined them for the purpose, consider that the scale and quality of effort directed to the provision of management services in Whitehall compare favourably with standards outside: Departments with nation-wide organizations appear to be conducted with real appreciation of the scope for application of management services.

The report deals, first, with the system of making decisions about Government expenditure; next, with the responsibilities of the Departments and of the Treasury in handling expenditure; and thirdly, with the question of Parliamentary control; while a final section contains some concluding observations on the future management of public expenditure. The central problem is that of how to bring the growth of public expenditure under better control and contain it within such limits as the Government may think desirable. This is the matter to which the Plowden Committee devoted most attention, and it is important that it should be clearly understood that its report is concerned with the control of expenditure within the limits laid down by policy, and not with the use of finance to concert policy. In practice, the piecemeal decisions which the Plowden Committee criticizes can have the effect of confining or impeding policy, and the definite conclusion is reached that decisions involving substantial future expenditure should always be taken in the light of surveys of public expenditure as a whole, over a period of years, and in relation to the prospective resources. Admittedly, judgments of the relative importance of one kind of expenditure or another may differ, but much of the criticism of Government expenditure in the scientific and technological field would lose its force if more care were taken to provide public opinion with the means for sound, informed judgments.

The new situation is attributed to the social, political and economic changes of the past two decades. Supply expenditure is now 22 per cent of the gross national product, compared with 12 per cent in 1930 and 6 per cent in 1910, while the public sector of the economy as a whole now employs about one-quarter of the total labour force and public expenditure of all kinds represents about 42 per cent of the gross national product. Moreover, its increasing complexity, including the cost of advanced technological projects and scientific research, the financing of commercial risks that the private sector cannot take, aid of many kinds to under-developed countries and social insurance schemes of unprecedented scope, involve commitments several years ahead. Further, the Budget is now seen as a sophisticated process in which the instruments of taxation and expenditure are used to influence the course of the economy, and the main weight of Parliamentary and public pressure is for innovations and improvements which cannot be effected without increases in public expenditure.

To meet this situation, the Plowden Committee favours a reconstruction based on four elements: regular surveys of public expenditure as a whole, over a period of years ahead, and in relation to prospective resources, decisions involving substantial future expenditure being taken in the light of these surveys; the greatest practicable stability of decisions on public expenditure, so that long-term economy and efficiency throughout the public sector have the best possible opportunity to develop; improvements in the tools for measuring and handling public expenditure problems, including in particular major simplification of the form of estimates, modernization and clarification of the Exchequer accounts, and more widespread use of quantitative methods in dealing with these problems throughout the public service; and more effective machinery for taking collective decisions and the bearing of collective responsibility by Ministers on matters of public expenditure.

One advantage of stability in expenditure policy noted in the report is regarded as the most important of all, although often overlooked. Such stability encourages sound planning by the Departments before the Government decision is sought. There is less danger of projects being rushed to decision to take advantage of a favourable event without being first subjected to adequate financial examination or measurement against other projects or means of securing the same purpose. That, and failure to fit the project into a rational expenditure pattern, can lead to great waste, and the major proposals in the first two parts of the report are intended to strengthen the organization against faulty decisions of all these types. On that ground alone they deserve close study by the scientist, for it is frequently in situations that arise out of scientific advance or technological change that the Government is required to act quickly and effectively and in the face of what appears to be great urgency, fanned by Parliamentary and public opinion. Apart from improving the control of public expenditure, they could provide a real safeguard against such pressure leading to undue support in one sector of science or technology rather than another.

The Committee's proposals are also designed to lead gradually to greater public interest in the priorities of expenditure, to stimulate academic thought about it, and to greater awareness of the implications and choices involved in policies as they come forward. The Committee emphasizes, in conclusion, the importance of building up an informed public opinion on the whole subject. Public opinion

has probably now come to accept that public expenditure and private expenditure both have their place in the national life, and that what is required is a balance between them. There are differences of opinion as to where that balance should be, nor is the balance easy to find, for insufficient is known as to the relation of public expenditure to the issues of growth and solvency of the national economy. Any Government, however, may find it difficult to maintain whatever balance it believes to be right in the face of the pressures, always powerful, to increase public spending in the modern community; and the best system and most up-to-date techniques of control will only succeed if public opinion is actively stimulated and enabled to take a balanced view of the alternative uses of national resources that are put forward, and to bring to the consideration of problems of public expenditure and resources the same qualities of common sense and realism that the average household shows all the time in handling its own affairs.

As regards the tools of control of expenditure, proposals for a new lay-out for the Estimates have already been made by the Committee and these were published in a Treasury Memorandum included in the Third Report from the Estimates Committee for the Session 1960–61*. The Government has already accepted the Plowden Committee recommendation for these changes, and the present report merely directs attention to one feature of the new lay-out, which includes for the first time for each Vote a statement showing how the total is divided between the categories used in the analysis in the National Income and Expenditure Blue Book. The Treasury has also begun a review of the form of Exchequer accounts, but generally the Plowden Committee received the impression that insufficient study had been given both inside and outside Whitehall to the effect of the size and composition of public expenditure on the growth and solvency of the national economy, nor have clear criteria been established to permit a rational choice to be made between different kinds of expenditure.

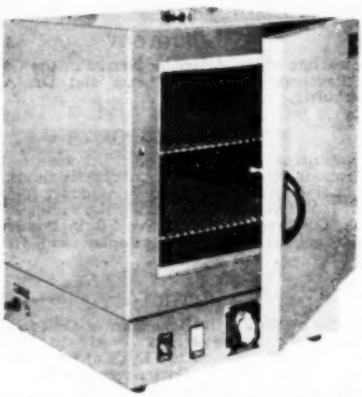
Developments envisaged in this report will call for a wider application of mathematical techniques, statistics and accountancy to problems of public expenditure, and there may also be scope for the use of the techniques generally described as operational research. Stress is laid on the advantage of seizing every opportunity for interchange of ideas and experience in management with people in industry. Further, since Parliament and public opinion no longer apply as systematic a restraint to public expenditure as they did in the past, the Plowden Committee suggests that some improvement is required in the arrangements to enable Ministers to discharge their collective responsibility for the review of public expenditure as a whole. Apart, however, from its stress on the importance of efficient management, the report makes some comment on Parliamentary control, though it does not go deeply into the relations between Parliament and the Executive in the control of expenditure. Here its main purpose is to show how Parliament's influence and authority can best be brought to bear constructively, and in this respect it is the complement of Basil Chubb's book under the same title, just as the major part of the report is a natural counterpart of Prof. S. H. Beer's study, *Treasury Control*.

There is thus much in this report that needs careful study by those concerned with the organization of Britain's research effort, whether in the purely scientific or the technological field. The report supports some of the proposals of the Select Committee on Estimates in its Sixth Report for the Session 1957–58, but it would be interesting to see the report of Sir Claude Gibbs's committee on managing and controlling research projects. The emphasis on the interlocking of public expenditure, for example, should be particularly noted, while the affirmation that in terms of administration it is unwise ever to attempt to make reductions or expansions in public expenditure by a process of equal percentage subtractions or additions all round will be warmly welcomed. Departments cannot sensibly be treated equally, and steady or falling expenditures require just as careful examination as those which may be rising.

In view of these pronouncements and the further emphasis on stability of expenditure, it is startling to find that the Council for Scientific and Industrial Research has suddenly, following the measures announced by the Chancellor of the Exchequer on July 25, suspended five major new grants that are imminent. In the statement making this announcement, which concerns the grants for the nuclear reactors for Scottish universities, and for the Universities of Liverpool and Manchester jointly, and the University of London, respectively, and for two radio telescopes for the Universities of Manchester and Cambridge, the Council expresses concern that, in view of the exceptional rate of expansion in numerous fields, the demands on its funds for 1962–63 are likely to exceed the planned resources. Until consideration of the detailed estimates for 1962–63 is complete, the Department is unable to enter into new major commitments.

The manner in which the announcement was made, however, suggests that an element of high politics has prevailed, all the more in that, as noted already, the Department has hitherto been almost unique among Government departments in using the system of estimates of expenditure for five consecutive years in advance. On the face of it, the Council, whether yielding to higher pressure or not, is departing from its own practice and flouting the unequivocal recommendation of the Plowden Committee. Much, of course, depends on how long the suspension proves to be. The projects are all the outcome of discussions between the universities and the Department, and it will not escape notice that meanwhile the radio-telescope for the Radar Research Establishment at Malvern is unaffected. Long delay with the projects could warp development in two fields of science in

* Third Report from the Estimates Committee together with part of the Minutes of Evidence taken before Sub-Committee A, and Appendix. Session 1960–61. Pp. vi+58. (London: H.M.S.O., 1961.) 4s. 6d. net.



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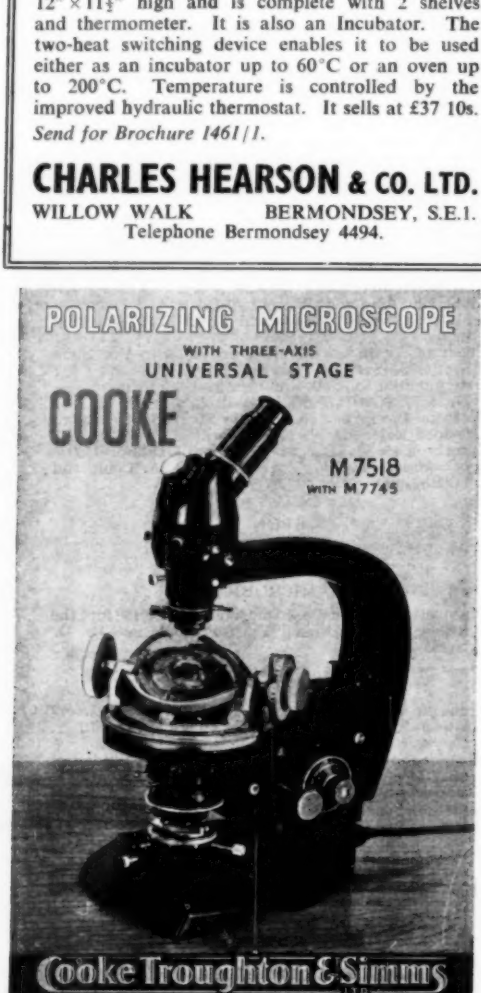

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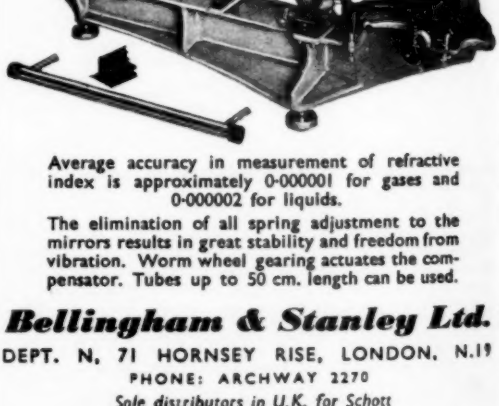

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CONTROL OF GOVERNMENT EXPENDITURE ON RESEARCH

THE Council for Scientific and Industrial Research, since its constitution some four years ago, has been concerned with the most effective means of budgetary control of scientific research in Britain. In its 1958 report the Council pointed out that, while it was possible to estimate research needs fairly accurately two years ahead, this could only be done provisionally five years in advance, and, accordingly, the Council sought as much flexibility as possible in allocating the funds at its disposal. A year later the Council stated that the Treasury had agreed that, without prejudice to the rest of the five-year plan to which the Council seeks to work, the Council might enter into commitments for research grants at a substantially higher level than was possible with the sums provided in the plan and that this level should be reviewed year by year.

In the Council's latest report, this policy was taken a stage further and the introduction was announced of an experimental scheme which should be more adaptable to rapid and unpredictable change. Under it the Council proposes each year to try to forecast its needs for five years in advance and to agree with the Treasury, subject to approval by Parliament, first the expenditure for the following year; secondly, reasonably firm estimates for the two succeeding years; and thirdly, less-definite estimates for the fourth and fifth years. This means that each year the estimates will be raised and brought forward one year, so that expenditure of each year will be planned against a fairly clear indication of probable expenditure in the years immediately following and with some regard to longer-term plans. Major departures from the estimates for the second and third years would only be made if there were important unforeseen changes in the scientific or financial situation.

This scheme seems to meet one comment made by the Select Committee on Estimates in its Fifth Report for the Session 1957–58, reviewing the work of the Department, when the Committee expressed the view that the timing of the termination of a piece of research could be as important as the timing of its initiation, and welcomed the Department's practice of regular reviews, not tied to the approval of the annual programmes, working independently of the advisory boards of the establishments. Strong support for this more flexible policy is now forthcoming from the Committee which, under the chairmanship of Lord Plowden, reported last June on the *Control of Public Expenditure**. This support is perhaps the more significant, first because although the Plowden Committee was not appointed to deal specifically with scientific or technical expenditure, it quickly distinguished the difficulties which characterize the control of such expenditure to-day; and secondly, because the difficulties encountered by the Council for Scientific and Industrial Research arise not simply from the mounting cost of scientific work but also from the effect of interlocking decisions taken elsewhere, as in educational policy, or in the fields of fuel, power, communications, space research, and the like.

Under the traditional system in Great Britain, as it developed in the nineteenth century and has continued throughout the lifetime of successive Governments to the present day, the tendency is for decisions on expenditure to be taken piecemeal. It is increasingly apparent that, where science and technology are concerned especially, this system is wasteful and liable to cause frustration and waste of resources of finance and material as well as of man-power. This is fully recognized by the Plowden Committee, and the core of its proposals is the development and use by Government of long-term surveys of expenditure and resources.

The report is, in fact, a State paper of outstanding significance which deserves—and indeed demands—close study by all who are concerned with the public finance of research and development and the most effective allocation of available resources. It is a sequel to an earlier report on Treasury Control of Expenditure from the Select Committee on Estimates in 1958, and the Committee was appointed in the summer of 1959. Several confidential reports have already been submitted to the Chancellor of the Exchequer, Mr. Selwyn Lloyd, and his predecessor, and the present report is a consolidation of those suitable for publication and discusses more especially the principles and practice of the control of public expenditure. It should stimulate exactly that constructive thinking and foresight which are imperative in dealing with many of the proposals for research and development projects to-day. In the judgment of the Committee, the crucial questions are not those of detail—the precise nature of the organization and chains of command within and between Departments, important though these are. The real problems are the wider issues of the purpose which the Government organization is attempting, its attitudes, what it regards as important, and its approach to its work on all matters involving public expenditure. Such issues are not always clear-cut, nor do they lend themselves readily to specific recommendations; but the scientist or administrator who reads this report side by side, for example, with the concluding passages of Sir Charles Snow's *Science and Government*, may find it a suggestive experience, for the whole report is designed to indicate fruitful lines of development in the future.

At the outset the Committee pays a handsome and deserved tribute to the work of the Civil Service which scientists should note. Changes, both in scope and character, in the activities of Government during the past two decades have produced strains in

* *Control of Public Expenditure*. Pp. 33. (Cmnd. 1432.) (London: H.M.S.O., 1961.) 1s. 9d. net.

traditional structures and practices, and Lord Plowden and his colleagues have been impressed by the way the Civil Service has adapted itself to deal with these changes. In particular, notable progress has been made in inter-departmental co-operation: there is a growing realization that in place of single departments dealing with individual problems in comparative isolation, the public interest requires a team pursuing its common objectives with a single purpose. This attitude of mind, and the readiness to initiate changes to improve control of expenditure, were manifested throughout the discussions between the Committee and the permanent secretaries and other officials of the major Departments. Furthermore, members of the Committee with experience of industry and commerce, and the specialist advisers who joined them for the purpose, consider that the scale and quality of effort directed to the provision of management services in Whitehall compare favourably with standards outside: Departments with nation-wide organizations appear to be conducted with real appreciation of the scope for application of management services.

The report deals, first, with the system of making decisions about Government expenditure; next, with the responsibilities of the Departments and of the Treasury in handling expenditure; and thirdly, with the question of Parliamentary control; while a final section contains some concluding observations on the future management of public expenditure. The central problem is that of how to bring the growth of public expenditure under better control and contain it within such limits as the Government may think desirable. This is the matter to which the Plowden Committee devoted most attention, and it is important that it should be clearly understood that its report is concerned with the control of expenditure within the limits laid down by policy, and not with the use of finance to concert policy. In practice, the piecemeal decisions which the Plowden Committee criticizes can have the effect of confining or impeding policy, and the definite conclusion is reached that decisions involving substantial future expenditure should always be taken in the light of surveys of public expenditure as a whole, over a period of years, and in relation to the prospective resources. Admittedly, judgments of the relative importance of one kind of expenditure or another may differ, but much of the criticism of Government expenditure in the scientific and technological field would lose its force if more care were taken to provide public opinion with the means for sound, informed judgments.

The new situation is attributed to the social, political and economic changes of the past two decades. Supply expenditure is now 22 per cent of the gross national product, compared with 12 per cent in 1930 and 6 per cent in 1910, while the public sector of the economy as a whole now employs about one-quarter of the total labour force and public expenditure of all kinds represents about 42 per cent of the gross national product. Moreover, its increasing complexity, including the cost of advanced technological projects and scientific research, the financing of commercial risks that the private sector cannot take, aid of many kinds to under-developed countries and social insurance schemes of unprecedented scope, involve commitments several years ahead. Further, the Budget is now seen as a sophisticated process in which the instruments of taxation and expenditure are used to influence the course of the economy, and the main weight of Parliamentary and public pressure is for innovations and improvements which cannot be effected without increases in public expenditure.

To meet this situation, the Plowden Committee favours a reconstruction based on four elements: regular surveys of public expenditure as a whole, over a period of years ahead, and in relation to prospective resources, decisions involving substantial future expenditure being taken in the light of these surveys; the greatest practicable stability of decisions on public expenditure, so that long-term economy and efficiency throughout the public sector have the best possible opportunity to develop; improvements in the tools for measuring and handling public expenditure problems, including in particular major simplification of the form of estimates, modernization and clarification of the Exchequer accounts, and more widespread use of quantitative methods in dealing with these problems throughout the public service; and more effective machinery for taking collective decisions and the bearing of collective responsibility by Ministers on matters of public expenditure.

One advantage of stability in expenditure policy noted in the report is regarded as the most important of all, although often overlooked. Such stability encourages sound planning by the Departments before the Government decision is sought. There is less danger of projects being rushed to decision to take advantage of a favourable event without being first subjected to adequate financial examination or measurement against other projects or means of securing the same purpose. That, and failure to fit the project into a rational expenditure pattern, can lead to great waste, and the major proposals in the first two parts of the report are intended to strengthen the organization against faulty decisions of all these types. On that ground alone they deserve close study by the scientist, for it is frequently in situations that arise out of scientific advance or technological change that the Government is required to act quickly and effectively and in the face of what appears to be great urgency, fanned by Parliamentary and public opinion. Apart from improving the control of public expenditure, they could provide a real safeguard against such pressure leading to undue support in one sector of science or technology rather than another.

The Committee's proposals are also designed to lead gradually to greater public interest in the priorities of expenditure, to stimulate academic thought about it, and to greater awareness of the implications and choices involved in policies as they come forward. The Committee emphasizes, in conclusion, the importance of building up an informed public opinion on the whole subject. Public opinion

has probably now come to accept that public expenditure and private expenditure both have their place in the national life, and that what is required is a balance between them. There are differences of opinion as to where that balance should be, nor is the balance easy to find, for insufficient is known as to the relation of public expenditure to the issues of growth and solvency of the national economy. Any Government, however, may find it difficult to maintain whatever balance it believes to be right in the face of the pressures, always powerful, to increase public spending in the modern community; and the best system and most up-to-date techniques of control will only succeed if public opinion is actively stimulated and enabled to take a balanced view of the alternative uses of national resources that are put forward, and to bring to the consideration of problems of public expenditure and resources the same qualities of common sense and realism that the average household shows all the time in handling its own affairs.

As regards the tools of control of expenditure, proposals for a new lay-out for the Estimates have already been made by the Committee and these were published in a Treasury Memorandum included in the Third Report from the Estimates Committee for the Session 1960–61*. The Government has already accepted the Plowden Committee recommendation for these changes, and the present report merely directs attention to one feature of the new lay-out, which includes for the first time for each Vote a statement showing how the total is divided between the categories used in the analysis in the National Income and Expenditure Blue Book. The Treasury has also begun a review of the form of Exchequer accounts, but generally the Plowden Committee received the impression that insufficient study had been given both inside and outside Whitehall to the effect of the size and composition of public expenditure on the growth and solvency of the national economy, nor have clear criteria been established to permit a rational choice to be made between different kinds of expenditure.

Developments envisaged in this report will call for a wider application of mathematical techniques, statistics and accountancy to problems of public expenditure, and there may also be scope for the use of the techniques generally described as operational research. Stress is laid on the advantage of seizing every opportunity for interchange of ideas and experience in management with people in industry. Further, since Parliament and public opinion no longer apply as systematic a restraint to public expenditure as they did in the past, the Plowden Committee suggests that some improvement is required in the arrangements to enable Ministers to discharge their collective responsibility for the review of public expenditure as a whole. Apart, however, from its stress on the importance of efficient management, the report makes some comment on Parliamentary control, though it does not go deeply into the relations between Parliament and the Executive in the control of expenditure. Here its main purpose is to show how Parliament's influence and authority can best be brought to bear constructively, and in this respect it is the complement of Basil Chubb's book under the same title, just as the major part of the report is a natural counterpart of Prof. S. H. Beer's study, *Treasury Control*.

There is thus much in this report that needs careful study by those concerned with the organization of Britain's research effort, whether in the purely scientific or the technological field. The report supports some of the proposals of the Select Committee on Estimates in its Sixth Report for the Session 1957–58, but it would be interesting to see the report of Sir Claude Gibbs's committee on managing and controlling research projects. The emphasis on the interlocking of public expenditure, for example, should be particularly noted, while the affirmation that in terms of administration it is unwise ever to attempt to make reductions or expansions in public expenditure by a process of equal percentage subtractions or additions all round will be warmly welcomed. Departments cannot sensibly be treated equally, and steady or falling expenditures require just as careful examination as those which may be rising.

In view of these pronouncements and the further emphasis on stability of expenditure, it is startling to find that the Council for Scientific and Industrial Research has suddenly, following the measures announced by the Chancellor of the Exchequer on July 25, suspended five major new grants that are imminent. In the statement making this announcement, which concerns the grants for the nuclear reactors for Scottish universities, and for the Universities of Liverpool and Manchester jointly, and the University of London, respectively, and for two radio telescopes for the Universities of Manchester and Cambridge, the Council expresses concern that, in view of the exceptional rate of expansion in numerous fields, the demands on its funds for 1962–63 are likely to exceed the planned resources. Until consideration of the detailed estimates for 1962–63 is complete, the Department is unable to enter into new major commitments.

The manner in which the announcement was made, however, suggests that an element of high politics has prevailed, all the more in that, as noted already, the Department has hitherto been almost unique among Government departments in using the system of estimates of expenditure for five consecutive years in advance. On the face of it, the Council, whether yielding to higher pressure or not, is departing from its own practice and flouting the unequivocal recommendation of the Plowden Committee. Much, of course, depends on how long the suspension proves to be. The projects are all the outcome of discussions between the universities and the Department, and it will not escape notice that meanwhile the radio-telescope for the Radar Research Establishment at Malvern is unaffected. Long delay with the projects could warp development in two fields of science in

* Third Report from the Estimates Committee together with part of the Minutes of Evidence taken before Sub-Committee A, and Appendix, Session 1960–61. Pp. vi+58. (London: H.M.S.O., 1961.) 4s. 6d. net.

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which Britain has established a leading position. It is, in any event, difficult to reconcile the announcement with the need to consider long-term requirements as well as the present situation which the Plowden Committee rightly stresses. An early examination of the position by, and an authoritative statement from, the Minister for Science, who apart from this should be studying the Plowden Report with some care and urgency, seem imperative.

That is the more important if the informed public opinion on which the Plowden Committee rightly insists is to be built up, for full and accurate information is an essential basis for public confidence and sound judgment. The point is the more significant in that in what is probably the weakest passage of the report, the Plowden Committee swings right against its concluding emphasis on informed public opinion and appears to endorse a policy of withholding from publication the long-term surveys of expenditure, prospective development of income or economic resources. Unless the maximum information is supplied, informed discussion of the issues is impossible: neither the perspective nor the basis for sound judgment is available, and Lord Hailsham might well reflect as regards the recent announcement by the Council for Scientific and Industrial Research, that in the case of departures from accepted policy, frankness and full publicity are the more essential. Secrecy is no basis either for confidence or sound judgment.

PARASITES OF MAN

Introduction to Parasitology, with special reference to the Parasites of Man.

By the late Prof. Asa C. Chandler and Prof. Clark P. Read. Tenth edition. Pp. xii + 822. (New York and London : John Wiley and Sons, Inc., 1961.) 78*s*.

THE appearance of yet another edition of this well-known book will be welcomed by all parasitologists, but their welcome will be greatly saddened by the knowledge that Asa Chandler, whose long life was devoted to the study of parasites and to the alleviation of the suffering caused by them to man and other animals, died while he was in the act of preparing this new edition. His great achievements in research and the devoted service he gave to human and veterinary medicine, and also to biology in general, will always be remembered by workers in these fields all over the world, for Chandler laboured, and was well known, in many countries, and the long list of his publications proves not only the width of his biological interests but also the practical value of the detailed work he accomplished.

In 1918 he embodied his knowledge and experience in a book entitled *Animal Parasites and Human Disease*, the fourth edition of which was, in 1930, rewritten to provide a general introduction to parasitology ; and the value of this second work was proved by the demand for further editions of it, its title being changed, in 1940, to its present form. Now, in this tenth edition, we are given the revision prepared by Prof. Clark Read, who has succeeded Prof. Chandler in the chair of biology at the Rice Institute, Houston, Texas. Prof. Clark has, he tells us in his preface, taken over full responsibility for the chapters on the Protozoa and the helminths, that is to say, for 512 of the 796 pages of text, and he has rewritten the chapters on the arthropods with the aid of notes left by Prof. Chandler.

The result is a volume which fully maintains the high standard of the earlier editions. It deals primarily with the parasites of man, but references are also made to parasites of domesticated and other animals, especially to those which are important in one way or another to human life and affairs. A notable change, which some readers will perhaps regret, is the deletion of the chapter on spirochætes, these organisms being now treated in a chapter on arthropod-borne organisms other than Protozoa, which also deals with the viruses, bacteria, *Rickettsiae* and the group of organisms of uncertain systematic position, such as *Anaplasma*, *Eperythrozoon*, *Grahamella*, *Bartonella and Haemobartonella*. Systematics have never been emphasized in this book, though necessary classifications, not always those favoured by English workers, are given, together with keys for the identification of species. The book has, in fact, always been remarkable for its exposition, in lively but not abstruse language, of the principles of parasitology, though it has always contained at the same time a great deal of detail cunningly woven into the text. Prof. Clark has maintained this combination of attractive style and detail and the text is now as up to date as any book, in these days of very rapid advances in knowledge, can hope to be. The references appended to each chapter and the list given of journals in which advances of knowledge are recorded, add, as the good index and the abundant illustrations also do, to the value of the book. Prof. Clark is to be congratulated on his careful and competent completion of what must have been a difficult job. G. LAPAGE

TREES OF CONSEQUENCE

The Eucalypts

Botany, Cultivation, Chemistry, and Utilization. By A. R. Penfold and J. L. Willis. (World Crop Books.) Pp. xx + 551 + 61 plates. (London: Leonard Hill (Books), Ltd.; New York: Interscience Publishers, Inc., 1961.) 80*s*. net.

SOMEONE reviewing a recent world conference in Brazil on eucalypt-growing made the rather sombre observation that the Earth is becoming covered with eucalyptus trees. The first specimen known to science was collected less than 200 years ago at Botany Bay on Captain Cook's first voyage, and now plantations throughout the world are estimated to cover 3½ million acres. The award of a volume in the series of World Crop Books is a mark of recognition of this success-story. The genus owes its progress equally to its versatility and its utility. One species or another can be provided to suit a wide range of habitats, often growing with remarkable vigour and sometimes in places which other trees find discouraging.

The genus *Eucalyptus* is reckoned as having more than 500 recognizable species, of which many are of economic value. In discussing eucalypt as a crop-plant, it is essential that we be clear what species we are concerned with, and inevitably much of this book is taken up with botanical descriptions and means of distinguishing one species from another. The first few chapters contain an account of the

genus, the characters of stem, flower and fruit. This leads up to sub-division of the genus and classification of the species, in which the system devised by Blakely is adopted.

The central part of the book deals with the eucalypts as crop-plants, their cultivation, their introduction as a forest-crop in many countries, the diseases and pests to which they are subject. In discussing its uses, the authors acclaim the genus as the most valuable source of hardwood in the world, good for structural work, for furniture, for sporting goods (Can those forceful Australian cricket-bats be made of eucalyptus?), for plywood and for fuel. No less remarkable in the genus is the wide range of essential oils, on which the authors write with particular knowledge, and from which are derived such varied products as antiseptics, inhalants, perfumes, sheep-dips and stain removers.

In the last, and by far the longest, chapter an attempt is made briefly to describe each species, what it looks like, where found, what conditions it needs, and what it is good for. The book includes some maps to show where eucalypts grow, an extensive bibliography, a good subject index, and some plates of uneven merit. The authors write clearly and competently, handling in orderly fashion a great mass of information. This book will prove a valuable guide to all who have to do with the production or products of this great genus, the stature of which as a world crop seems certain to grow mightier yet.

T. A. Russell

COMPUTER DESIGN

Computer Logic

The Functional Design of Digital Computers. By Dr. Ivan Flores. (Prentice-Hall Electrical Engineering Series.) Pp. xii+458. (London : Prentice-Hall International, Inc., 1960.) 45*s*.

Design Fundamentals of Analog Computer Components

By Prof. R. M. Howe. Pp. x+268. (Princeton, N.J. : D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 7.50 dollars; 56*s*. 6*d*.

THE legend on the dust cover of the first of these books reads "How computers are put together—how they work—how to use them". This describes the book better than the title. It is not a text-book on computer design, but is addressed to the general scientific reader of whom a minimum is required in the way of engineering and mathematical background. Nevertheless, it contains a very solid treatment of logical design; the binary system, adders, multipliers, control units, input and output devices, magnetic tape, and so on, all will be found described here, together with the usual ration of Boolean algebra and formal logic. More attention is paid than in similar books to the mechanical details of input and output equipment.

Only a small part of the book is concerned with programming, but this part I found unsatisfactory. It seems a mistake, now that modifier registers are the order of the day, to introduce the reader first to the old-time procedure for modifying instructions in the arithmetic unit. The last chapter, on programming a particular scientific problem, is a veritable museum piece, and leads one to think that the author must have been entirely insulated from the development of programming techniques. It exhibits the most primitive form of machine language coding possible, in which even the conversion of relative addresses in sub-routines to absolute addresses must be done by the programmer himself before his programme is punched. In these days, even if a programmer does not use some form of automatic programming, he may expect to have the assistance of a more or less elaborate input programme which provides, among other facilities, automatic relative addressing. The book would have been better without the last chapter.

The purpose of the second book is to give users of present-day analogue computers some idea of how these machines came to be designed as they were. The author believes, quite rightly, that a complete understanding of the capabilities of an electronic analogue computer cannot be obtained without a considerable knowledge of the component details and how they affect the computer solutions. The book contains chapters on systems design, component accuracy, d.c. amplifiers, multipliers, function generators, recorders and miscellaneous equipment. An important and valuable feature of the book is the large amount of detailed and comparative design information given about some of the better-known commercially available analogue computers. The book is very well written, and those not themselves working in the analogue field may find it interesting as showing the remarkable way in which analogue computers have developed from the primitive devices that they were ten years ago to their modern form.

M. V. Wilkes

THE BACTERIAL CELL

Synthesis and Organisation in the Bacterial Cell

By E. F. Gale. (Ciba Lectures in Microbial Biochemistry, 1959). Pp. vii+110. (New York and London: John Wiley and Sons, Inc., 1959.) 3.50 dollars, 28*s*.

ACCORDING to *The Times* of August 14, the National Union of Students reported in a memorandum to the Committee on University Teaching Methods that they have received complaints of lectures in universities being ill-prepared, badly presented and giving only what is already available in books. One wonders to what extent these criticisms apply to teachers of biochemistry. Biochemical research is so prolific and occasionally so disturbing even to basic concepts that an unrevised text-book more than ten years old is scarcely more useful to a final honours student than last term's cell-free bacterial extract. It is possible that there exist lecturers who fear that "to be intelligible is to be found out"[1], but the same writer also affirmed that "the truth is never pure and rarely simple"[2].

These three eminently intelligible lectures by Dr. Ernest F. Gale are concerned with synthesis and organization in the bacterial cell, the truth about which grows increasingly complicated; but he selects, discusses and condenses into a small volume those findings from a vast accumulation of recent research publications which are of real significance. The style is lucid, the topics stimulating and the book beautifully presented: thus, all readers will admire the electron micrograph of Plate I and the fluorescent

micrograph of Plate III. These lectures are a model of how to tackle one difficult task of a lecturer to final-year students, although no doubt such students made up only a very small proportion of his American audience.

This task was to re-assess the findings of ten years ago in the light of those made in the past two. For example, Dr. Gale shows that although many experiments concerned with amino-acid incorporation into disrupted *Staphylococci* contributed to our present knowledge of protein biosynthesis, some of the results were bewildering. Now, they may be interpreted readily since knowledge of the chemical nature of the cell wall has advanced and it is realized that certain amino-acids may be incorporated into substances other than true protein. The lecture reported in Chapter 1 deals with the structure of the bacterial cell, with an account of the cell wall and membrane, and Chapter 2 deals with amino-acid incorporation. Chapter 3, on nucleic acid and protein synthesis, is stimulating but the data are already in need of a second reappraisal. Since the lecture was delivered, the ribosomes in which bacteria are so rich and which excited so much interest a few years ago seem no longer to hold their central place in metabolism as templates for protein synthesis. "Ribosomes are non-specialized structures which synthesize, at a given time, the protein dictated by the message they happen to contain"[3]. As a consequence of the discovery of this type of RNA called 'messenger', Dr. Gale's convenient nomenclature of rna and RNA, according to the size of the molecule, will need revision. It is certain that present discoveries will lead to a tremendous spate of new publications, some of which will no doubt appear to be contradictory, and it is to be hoped that Dr. Gale will be persuaded to survey the field of protein biosynthesis again in about a year's time if only to lighten the burden of the harassed lecturer and his critical, if not angry, young audience.

The book contains few errors. A glucosyl residue in Fig. 2 lacks a hydroxymethyl group; while the apt quotation from Sir Thomas Browne omits whales from the list of prodigious pieces of Nature given in the first authentic edition of *Religio Medici* (1643) and states that the civility of the little citizens more nearly ('more neatly' is correct) "sets forth the wisdome of their Maker".

S. Dagley

[1] Wilde, O. O'F., *Lady Windermer's Fan*, Act 1 (1892).
[2] Wilde, O. O'F., *The Importance of Being Earnest*, Act 1 (1895).
[3] Brenner, S., Jacob, F., and Meselson, M., *Nature*, **190**, 576 (1961).

POLYMER CHEMISTRY

Preparative Methods of Polymer Chemistry

By Wayne R. Sorenson and Tod W. Campbell. Pp. viii+337. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 79*s*.

THE polymer chemist in search of a good method for the laboratory preparation of a particular polymer has in the past been obliged in many cases to carry out a tedious and time-consuming search through *Chemical Abstracts*, all too often with the frustrating result that the desired information is contained in an inaccessible journal. The appearance of a laboratory handbook which collects details of the great majority of polymer-forming reactions and preparative data for a very wide range of polymers will therefore be noted with interest and probably also relief by workers in all branches of this subject.

This cannot have been an easy book to write. The field to be covered is very extensive indeed, it is expanding rapidly, and its sub-division into types of polymer or reaction is not always straightforward. Experimental methods in the literature range from the most meticulous high-vacuum techniques with highly purified materials down to the crudest 'cooking together' in air of commercial-purity reagents, so that the authors must have devoted a lot of thought to selecting the most suitable standards of procedure to adopt in a book of this type. There exists also the difficult problem of the reliability of various published preparations—How is this to be judged? It is therefore greatly to the authors' credit that satisfactory solutions to all these problems have been found.

After a short introductory chapter, some fifty pages are devoted to experimental techniques for the preparation, fabrication and characterization of polymers. The remainder of the book consists of detailed methods of preparation, divided into chapters headed "Polycondensation and Hydrogen Transfer Reactions", "Addition Polymers from Unsaturated Monomers", "Ring Opening Polymerization", "Non-classical Routes to Polymers", and "Synthetic Resins". Each sub-section, containing a number of representative preparations, is preceded by a brief theoretical introduction outlining the principles and mechanisms involved, and giving numerous references to sources of more detailed theoretical treatment. The experimental procedures, although mainly directed to polymer preparation, include quite a few well-chosen examples of reactions on polymers.

The book has many features which deserve favourable comment. The importance of purity of materials is stressed in Chapter 2 and again on numerous occasions throughout the book. The potential danger in many polymerizations is emphasized repeatedly, and where any particular preparation involves special hazard from the type of reaction or the poisonous nature of the materials, this is clearly stated in bold type. The book is very comprehensive, but on those occasions where topics are considered outside its scope, references to other sources of information are given. The lists of references at the ends of the chapters are most extensive, totalling more than five hundred. The methods included are of a high enough standard (with few exceptions) to satisfy everyone but the most demanding research worker, who can easily modify the procedures to suit himself. The details given in the preparations are not the over-abridged accounts which appear in some of our more space-conscious journals, but include numerous helpful comments. The information is thoroughly up to date; few of the latest types of polymer-forming reaction are omitted, except (understandably) in the field of inorganic polymers. In addition to a very good general index, there are indexes by initiator system, reaction system and functional groups. I was impressed also by the clear type, attractive layout and good binding. There are very few misprints.

This is a book which most polymer chemists would find useful. It would also be helpful in connexion with undergraduate courses on practical methods of polymer chemistry. It is an important addition to polymer literature which I recommend highly.

I. C. McNeill

Proceedings of the Natural Rubber Research Conference

Kuala Lumpur, 1960. (Sponsored by the Rubber Research Institute of Malaya, with the support of the Government of the Federation of Malaya and the Rubber Producers' Council of the Federation of Malaya.) Pp. x+986+xix. (Kuala Lumpur: Rubber Research Institute of Malaya, 1961.) 53*s*. 8*d*.

THIS volume constitutes a comprehensive review of present-day research relating to natural rubber. From the opening address the conference faced up to progress in competitive synthetic rubbers and showed from the papers collected in this volume the impressive and many-sided research activities designed to maintain and expand natural rubber production.

The longest section, 436 pages, deals with scientific study of *Hevea brasiliensis* with the view of encouraging it to ever greater production of latex. Several papers in each sub-section deal with nutrition of the tree, stimulation of latex yield on tapping, ground cover and weed control on the plantation, breeding and selection, pests and diseases. A shorter section follows on the production of dry rubber with more stable properties and on modifying its properties at the latex stage or in the manufacturer's factory; a paper herein on factory processing of synthetic *cis*-polyisoprene indicates an absence now of an ostrich-like attitude towards such a synthetic competitor. Chemistry and technology of latex occupy most of the remainder of the book.

The book is likely to be found by botanists and biochemists to have interest in the individual reports themselves, and as an object lesson in the concerted effort on the one plant both from many disciplines and from many countries. Larger libraries collecting the literature of rubber will no doubt obtain it and many rubber technologists will find it interesting to browse through. W. F. WATSON

Elements of Functional Analysis

By L. A. Liusternik and V. J. Sobolev. Translated from the Russian by Anthony E. Labarre, Jr., Herbert Izbicki, and H. Ward Crowley. Pp. ix+227. (New York: Frederick Ungar Publishing Company; London: Constable and Co., Ltd., 1961.) 55*s*. net.

IN 1936, Liusternik wrote a report on fundamental concepts in functional analysis. His summary of methods and results was expanded by Sobolev into a course of lectures, and the resulting account of the elements of functional analysis grew into the Russian edition of this book. The date of the Russian edition is not stated; most of it has the air of having been written fifteen or twenty years ago, and the general style and outlook is similar to that of Gelfand, Raikov and Shilov in their work on normed rings (about 1946) which at the time led many mathematicians to appreciate the power of the new methods. There is an isolated reference to a paper published in 1958 and a few others to publications of the late 1940's.

The first four chapters are devoted to spaces and operators. They are clear and concise, and as useful an account as can be found anywhere of what every young functional analyst should know. The leading ideas are necessary not only to the functional analyst but also for research in almost any field. Chapter 5 introduces the reader to the methods of spectral theory by bounded self-adjoint operators; this will form a good background to the large amount of recent work. The mention of non-linear analysis in the title of Chapter 6 leads the reader to expect interesting and important developments, but the chapter is mainly concerned with rather heavy theorems on implicit functions and the calculus of variations. J. C. BURKILL

Theoretical and Experimental Physics

By Dr. H. G. Jerrard and Dr. D. B. McNeill. Pp. xiv+624+6 plates. (London: Chapman and Hall, Ltd., 1960.) 75*s*. net.

THIS book is primarily a manual of laboratory work in physics, but has the advantage that it also contains a considerable amount of the relevant theory and about fourteen hundred useful references as well. It should be of great value to university students who are studying physics to the pass-degree level and also, in parts, to those taking an honours course in physics.

Chapters 1 and 2 are essentially an introduction to the remainder of the book. The former has three sections dealing with the representation of experimental results by means of graphs, experimental errors and units (including rationalized M.K.S. units). Chapter 2 is a very useful one and is devoted to a general description of some of the standard apparatus and equipment used in a modern physics laboratory; it is divided into three parts, dealing with optical, electrical and more general apparatus.

The remaining chapters, 3–20, then describe experiments in mechanics, properties of matter, heat, acoustics, geometrical optics, physical optics, electricity, electronics and magnetism. The presentation in these chapters is a very novel one. The right-hand pages contain a description of the experiments while the left-hand pages contain, in smaller type, material of a more advanced nature meant to amplify the work described on the right-hand pages. The material on the left-hand pages may be omitted if the reader so desires.

The appearance of the book is to be welcomed and it should prove stimulating to both students and to those who teach them. D. H. TREVENA

Bibliography and Index of Geology and Allied Sciences for Wales and the Welsh Borders

1897–1958. By D. A. Bassett. Pp. 376. (Cardiff: National Museum of Wales, 1961.) n.p.

THIS bibliography covers works on geology and allied sciences that either deal specifically with Wales and the Welsh Border country, or contain references to the geology of these regions. The period covered is 1897–1958. Earlier works are listed in several published bibliographies, the titles of which are given by the compiler.

The book is divided into a number of sections, the principal ones being a list of books, papers, etc., an author-index, and a subject-index. There is also a useful section listing unpublished higher degree theses on the geology of Wales. The first of these sections is further subdivided chronologically into sections for each year. This does not make for ease of reference if, for example, one wishes to consult all the works of a single author. Again, the compilation of a subject-index is a task which must inevitably be affected by the personal equation. This one is no exception, and it would be easy to fault it. As a whole this bibliography seems somewhat over-elaborate, but nevertheless as a work of reference it will undoubtedly prove valuable to all workers in the field of Welsh geology. V. A. EYLES

XUM

A SURVEY OF NEW PENICILLINS

By PROF. ROBERT KNOX
Guy's Hospital Medical School, London, S.E.1

SINCE Fleming's discovery of penicillin in 1929[1] and its later development by Florey and Chain and their collaborators[2] many new antibiotics have been discovered, some of them active against organisms not affected by penicillin, others with a broader spectrum of antibacterial activity. But penicillin (benzyl penicillin or penicillin G) is still in many ways the best. It is effective in minute doses against sensitive organisms, is often bactericidal, almost completely non-toxic, and does not show the rapid development of resistant mutants often seen with other antibiotics such as streptomycin. However, penicillin G is rapidly excreted and comparatively ineffective by mouth; it is liable to give rise to hypersensitivity reactions, sometimes fatal, and the range of its activity is limited—for example, many Gram-negative organisms are insensitive to it. Although most species of organisms do not become rapidly resistant to penicillin G either *in vitro* or *in vivo*, penicillinase-producing staphylococci all over the world have become an increasingly serious menace, especially in hospitals.

For many years it has been known that different types of penicillin were produced by *Penicillium chrysogenum* when cultural conditions were varied. When appropriate precursors were added to the fermentation liquor, as in the production of benzyl penicillin, chemical and microbiological assays of the penicillin formed were in agreement; but when the precursor was omitted from the fermentation liquor a substance was formed which gave the chemical reactions of a penicillin but had much less antibacterial activity. Kato[3] suggested that this was the penicillin nucleus which Sakaguchi and Murao[4] had claimed could be obtained by splitting benzyl penicillin with an amidase. Sheehan[5] reported that he had synthesized the nucleus, 6-aminopenicillanic acid, but the chemical synthesis was difficult and the yields were low. In 1959, Batchelor, Doyle, Nayler and Rolinson[6], at the Beecham Research Laboratories, isolated pure 6-aminopenicillanic acid from fermentation liquors to which no precursor had been added[7–12]. This was a discovery of the first importance and has deservedly been recognized as a major step forward in chemotherapy, both for its practical results and for its theoretical interest. 6-Aminopenicillanic acid could now be obtained in high yield from fermentation liquors with no added precursor. As a result of this work new penicillins are being produced at bewildering speed. Some of these represent important advances in chemotherapy, others are not quite in that class but may have real advantages in special conditions, while others are interesting more because of their theoretical possibilities or because they may serve as prototypes for better models in the future. But there has been a good deal of overlapping and confusion and in some cases the differences are only marginal. It is therefore worth while to survey the present situation and to consider what in fact has been achieved, what reasons there are for expecting further achievements in the future and what light these penicillins may throw on the mode of action of penicillin and the origins of penicillin resistance.

The New Penicillins

It was early realized that there were many penicillins which showed differences in antibacterial activity, for example, penicillin X (*p*-hydroxybenzyl) or K (*n*-heptyl penicillin). But it was soon accepted that the most active for general purposes was benzyl penicillin or penicillin G. At that time when we referred to the 'penicillin' type of antibacterial spectrum we meant the spectrum of penicillin G.

From 1953 onwards penicillin V or phenoxymethyl penicillin came into use[13–15]. It is acid-stable and effective by mouth. It has much the same spectrum of antibiotic activity as penicillin G, but there are differences, some of which may be important.

The first 'synthetic' penicillin was phenoxyethyl penicillin or phenethecillin ('Broxil', 'Syncillin')[16–18]. Like penicillin V it is effective by mouth. The relative merits of phenethecillin and penicillin V have been disputed. Phenethecillin gives higher blood levels than penicillin V, but against most sensitive species of organisms its *in vitro* activity is less. However, phenethecillin is more active *in vitro* than either G or V against some strains of penicillinase-producing staphylococci[19–24].

Shortly after this the discovery of methicillin ('Celbenin')[25–34] was announced. Methicillin was the first penicillin with considerable activity against large inocula of both penicillin G-sensitive and resistant staphylococci. The value of this discovery can scarcely be exaggerated. Methicillin has found an accepted place in the treatment of resistant staphylococcal infections[35]. It has also great theoretical interest. Besides being highly resistant to the action of staphylococcal penicillinase, it is a powerful inducer of the enzyme. Staphylococci are thus stimulated to form the enzyme by an inducer which they cannot use as a substrate and therefore cannot destroy. Methicillin, however, has its defects. Although adequate blood levels can be obtained its antibacterial activity against staphylococci is of the order of 1–4 μgm./ml. as compared with about 0·02 μgm./ml. for the activity of penicillin G against sensitive staphylococci. Methicillin is not effective by mouth and injections are sometimes very painful, though this difficulty can be overcome[35].

Phenoxypropyl penicillin (*PA* 248, Pfizer, Ltd.)[36] was reported to be slightly more active *in vitro* than phenethecillin against penicillinase-producing staphylococci. But it does not approach methicillin in activity against large inocula of these. Against other coccal infections it seems to have about the same activity as other oral penicillins such as penicillin V or phenethecillin.

Recently, new ground has been broken by the announcement that 6[D(−)α-aminophenylacetamido] penicillanic acid or α-aminobenzyl penicillin ('Penbritin')[37–40] has been synthesized at the Beecham Research Laboratories and tested experimentally

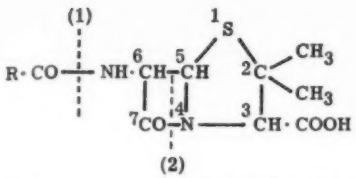

Fig. 1. General formula of a penicillin. (1) Site of action of penicillin amidase (splitting the amide linkage). (2) Site of action of penicillinase (giving rise to penicilloic acid)

and clinically. It shows considerable activity against many organisms against which penicillin G is relatively ineffective, including many of the Gram-negative coliform organisms such as *E. coli* itself, *Proteus*, *Salmonella*, *Shigella* and *H. influenzae*. It is thus the first broad-spectrum penicillin and is therefore of great practical and theoretical importance[41]. Clinically it promises to be useful in the treatment of urinary infections and chronic bronchitis and in many gastro-intestinal infections, especially typhoid fever. Its spectrum of activity is similar to that of the tetracyclines and chloramphenicol. It has considerable activity against the cocci and other organisms naturally sensitive to penicillin G.

The penicillins so far discussed have all shown sufficient promise to justify their commercial production for clinical use. Two other substances should be mentioned here because of their great theoretical interest and importance. 6-Aminopenicillanic acid itself, the penicillin nucleus, is the starting point for the synthesis of many new penicillins, either chemically or by means of amidases which occur in many species of micro-organisms. Amidases can also hydrolyse penicillins into the free side-chain and 6-aminopenicillanic acid[42,43]. This type of action is quite distinct from the opening of the β-lactam ring which occurs when 6-aminopenicillanic acid is acted on by penicillinase. These two types of enzymic action are shown in Fig. 1.

Cephalosporin C [44] has a nucleus, 7-aminocephalosporanic acid, which consists of a β-lactam ring, fused, not with a thiazolidine ring as in 6-aminopenicillanic acid, but with a 6-membered sulphur-containing dihydrothiazine ring. Cephalosporin C and related substances have some of the properties of penicillins. Cephalosporin C itself like methicillin is very stable to staphylococcal penicillinase and is also a powerful inducer of the enzyme. But its antibacterial activity is very low. Although recently considerably more active derivatives of cephalosporin C have been obtained the activity is still less than that of penicillin G.

Some of the main characteristics of these penicillins are summarized in Table 1.

Different 'Functions' of Penicillin

There are at least seven 'functions' or activities of a penicillin to be considered—toxicity, activity in the presence of protein, acid-stability, production of hypersensitivity, stability to penicillinase, ability to induce penicillinase and finally antibacterial activity. To be of real clinical value, a penicillin must if possible be non-toxic, active in the presence of protein, capable of producing adequate levels in blood in therapeutic doses, and, if it is to be given by mouth, acid-stable. The new commercially produced penicillins have all these properties—except methicillin, which is ineffective by mouth. So far as is known, patients hypersensitive to one penicillin are likely to be hypersensitive to another, but more clinical and experimental evidence is needed. It is not even certain how frequent hypersensitivity to the older penicillins really is. Some reports have suggested that allergic reactions, many of them serious, are becoming more frequent[45]. A recent survey[46], however, shows that of 35,496 patients attending clinics for penicillin injections only 255 (7·2 per 1,000) showed reactions, and only 9 patients showed moderate or severe 'anaphylactic' reactions. But the frequency of reactions increased up to 66 per 1,000 in patients receiving large doses for several days.

Antibacterial Activity, Penicillinase Stability and Induction of Penicillinase in Different Penicillins

The three functions of antibacterial activity, of stability to penicillinase and of capacity to induce penicillinase can to some extent be segregated in different penicillins[47]. This segregation gives the facts on which some useful working hypotheses may be based. It will be useful to start by comparing the antimicrobial activity of these different penicillins in terms of their stability to penicillinase (Table 1). With staphylococci sensitive to penicillin G it seemed at first as though the more easily a penicillin was split by penicillinase the greater was its antibacterial activity. But the correlation no longer holds with α-aminobenzyl penicillin, which is much more easily hydrolysed by penicillinase than penicillin V or G, yet rather less effective against sensitive staphylococci. With penicillinase-producing staphylococci, at first sight it seems that the more resistant it is to penicillinase the more effective will a penicillin be. With large inocula this is true, but not with small. Small, and sometimes even moderate, inocula of these are

Table 1

MAIN FEATURES OF DIFFERENT PENICILLINS

Structural Formula R =	Name	Antibacterial Activity: Gram+ cocci.	Penase+ staph.	Gram neg. rods	Hydrolysis by staphylococcal penicillinase	Induction of staphylococcal penicillinase
CH_2-CO-	Benzyl, G	+++	−	(±)	++	+
$O-CH_2-CO-$	Phenoxymethyl, V	+++	−	−	++	+
$O-CH(CH_3)-CO-$	Phenoxyethyl (phenethecillin, broxil, syncillin)	+++	−	(±)	−	++	++
$O-CH(CH_2CH_3)-CO-$	Phenoxypropyl (PA 248)	+++	−	(±)	−	++	++
2:6 dimethoxyphenyl $-CO-$	2:6 dimethoxyphenyl (methicillin, celbenin, staphcillin) (BRL 1241)	+	+	−	−	++
$CH(NH_2)-CO-$	α-aminobenzyl (BRL 1341)	++	−	+	+++	+
—	6-aminopenicillanic acid	(−)	(−)	(−)	(±)	(±)

more sensitive to phenethecillin or phenoxypropylpenicillin than to either penicillin G or methicillin, despite the fact that both are slightly more stable than penicillin G and very much less stable than methicillin to staphylococcal penicillinase. A penicillin stable to one penicillinase is not necessarily stable to others. Methicillin is only slowly attacked by staphylococcal penicillinase but is more vulnerable to *B. cereus* penicillinase and probably to penicillinases from some strains of *Proteus*[48] (though it has not yet been proved whether destruction of methicillin by *Proteus* is due to an amidase or a penicillinase). The only highly active 'Gram-negative' penicillin so far available, α-aminobenzylpenicillin, is the most susceptible of all to staphylococcal penicillinase[47].

There is a closer correlation between the stability of a penicillin to penicillinase and its capacity to induce the enzyme in that species (see Table 1). With 6-aminopenicillanic acid itself both the rate of hydrolysis by staphylococcal and *B. cereus* penicillinase and its ability to induce enzyme formation seem to vary considerably with the conditions used. For example, Steinman's[49] figures do not agree with those of Batchelor *et al.*[11], who found that the *p*H for maximal speed of hydrolysis was different for 6-aminopenicillanic acid and penicillin G, and that at their respective *p*H optima both substrates were split at much the same rate. As regards inducing ability, it is necessary to distinguish between the concentration required for maximal or half-maximal induction and the total amount of enzyme finally formed. But while the relative 'efficiency' of 6-aminopenicillanic acid and penicillin G as inducers of penicillins is likely to depend greatly on the conditions used, for example, *p*H, evidence so far available[47,49] suggests that neither is as good an inducer as methicillin.

Structural Basis for the Activity of Different Penicillins

With several enzymes, notably succinic dehydrogenase[50–52] and cholinesterase[53], a good deal is known about the relation of molecular structure to specificity of action. Similarly, the new penicillins are already beginning to give a clearer picture of the relation between the different aspects of penicillin activity. Considering first the enzyme-substrate aspect, we may picture a penicillin molecule diagrammatically (Fig. 2) as having a number of specific groups which become anchored to complementary groups on the 'active patch' of the enzyme penicillinase. If the fit between the penicillin and the enzyme is good enough then carbon atom (7) is pulled away from nitrogen atom (4) (Fig. 1), the β-lactam ring is opened, the penicillin is hydrolysed, and another penicillin molecule takes its place in apposition to the active patch of the enzyme, and so the process continues. A penicillin resistant to penicillinase is one in which sufficiently close contact cannot be achieved for the sequence of reactions which result in the splitting of the β-lactam ring. This might be because other groups such as the two methoxy groups in methicillin cause steric hindrance, or, as in cephalosporin C, because the molecule is not quite of the right shape for the necessary contacts to be made with receptors on the enzyme surface all along its length. For induction, however, there may not be the need for 'multi-point' contact but only for 'anchoring' at a small number of key-points (*I* in Fig. 2). The immediate precursor of the penicillinase molecule or indeed perhaps the molecule itself may be regarded as having active groups on its peptide backbone corresponding to complementary groups on the penicillin molecule. One of these might be the C=O group at C (7); others may be at the ends of the penicillin molecule. In the uninduced enzyme the apposition between these complementary pairs of groups is inaccurate. The actual process of induction may consist of 'straining' the molecule of penicillinase (or enzyme-forming site)[54] so that now the corresponding groups on the enzyme and the penicillin can be anchored, perhaps by hydrogen bonds[51], perhaps in other ways. An inducer which is stable to penicillinase then would be one which can strain the enzyme molecule so that it is now capable of hydrolysing any penicillin which has the necessary groups on its molecule. But the penicillinase-stable inducer itself will not be hydrolysed because it lacks these or because other groups on its molecule prevent contact with the corresponding receptors on the enzyme molecule. The enzyme once induced will maintain its activity so long as it is presented with a penicillin —either with one which it can hydrolyse or with one which continues to maintain the enzyme in the induced state. Pollock[55] studied the capacity of different penicillins and penicillin derivatives to induce the formation of penicillinase in *B. cereus* and established some of the structural requirements for induction. The value of such comparisons will be greatly increased as new penicillins become available.

The interactions occurring at the specific site may be regarded then as involving two main steps. In the first, the inducer exerts on the pre-enzyme, on the enzyme-forming system, or on the actual enzyme the same forces that the substrate exerts on the enzyme (or the enzyme on the substrate). Thus both a substrate and inducer are, as it were, 'conditioned' for the next stage—the splitting of the β-lactam ring. But with an inducer which is not a substrate the actual splitting does not occur for the reasons already suggested.

It is less easy to construct a convincing model of the antibacterial activity of different penicillins. Cooper[56] has suggested that the process of attachment of penicillin to the receptor site in the penicillin-sensitive cell is associated with the opening of the β-lactam ring, in other words, with a penicillinase-like reaction. It is not known whether the receptor with which the penicillin reacts is a precursor of the enzyme penicillinase, or the enzyme itself, or a 'penicillin-binding component' or enzyme-forming system which reacts with the penicillin molecule in such a way that either the penicillin destroys the cell or the cell destroys the penicillin[55,56]. Whichever of these alternative theories we adopt it seems clear that similar if not identical receptors are involved in penicillinase induction and activity and in the antibacterial action of penicillin. I have already suggested that for efficient hydrolysis of a penicillin to occur by means of the enzyme penicillinase there must be 'multi-point' contact all along the molecule with the corresponding active centres on the enzyme surface. Similar, though not identical, closeness of fit may be necessary for antibacterial action, and the type of antibacterial action may be determined by the closeness of fit at different sites. Two of these seem to be especially important—the 'amide' carbon and the first carbon beyond it on the side-chain.

Fig. 2 represents diagrammatically the ways in which these different properties of a penicillin may

be related and suggests the following hypothesis. The capacity to induce is a property of all penicillins and it depends partly on the length of the molecule. When induction occurs the surface of the enzyme or the enzyme-forming system is strained so that it is brought into intimate contact with the two ends of the molecule and with carbon (7) in the β-lactam ring. The induced enzyme can be regarded as being under some sort of tension, and it is only when it is brought into intimate contact in this way with these key-points that the rest of the molecule is able to exert its hydrolytic effect. Fig. 2(1*a*) shows the situation that may exist with benzyl penicillin when it is brought into apposition with an active penicillinase. For maximal Gram-positive activity the area marked in the diagram must be brought into apposition with a cell 'receptor', which may be the same as a receptor on the enzyme surface; for maximal Gram-negative activity the area marked Gram-negative must be brought into apposition with the corresponding 'Gram-negative' area. The penicillinase-stable methicillin can still induce, but splitting of the β-lactam ring is impossible (Fig. 2(2)). There is still room for some Gram-positive activity with penicillins of this kind but the Gram-negative activity must necessarily be lost; in other words, the very steric hindrance which makes a penicillin of this kind resistant to penicillinase will make it ineffective against the Gram-negative organisms which have a receptor surface which must be brought into close contact with one of the side-chains attached to the carbon next to the aromatic ring. This activity is greater when one of the hydrogens in penicillin G is replaced by NH_2 (Fig. 2(1*b*)), which brings about close contact with the 'Gram-negative' site.

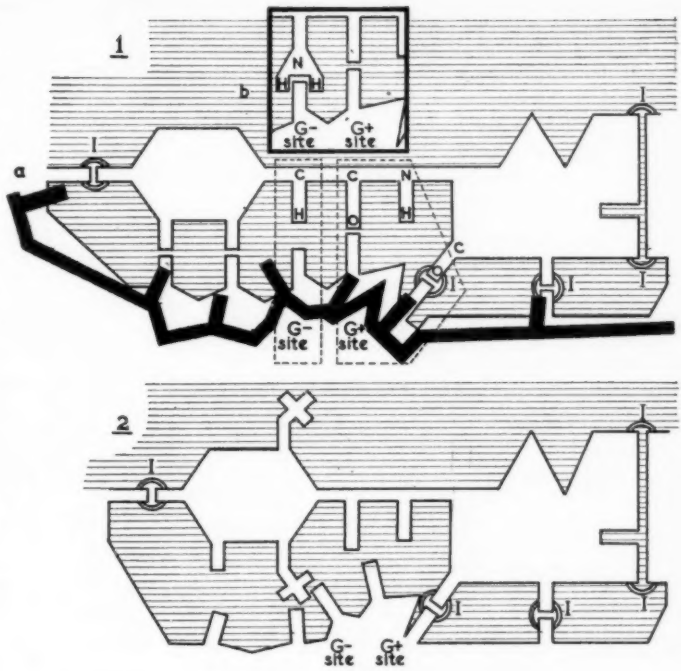

Fig. 2

At the cellular level, there have been many theories about the site of the action of penicillin. Many of the earlier observations can be explained in the light of recent evidence that penicillin interferes with the incorporation of amino-acids into the cell-wall and thus with the synthesis of cell-wall mucopeptide[57]. Rogers and Jeljaszewicz[58] have shown that the order of effectiveness of different penicillins is the same for both antibacterial activity and interference with cell-wall mucopeptide synthesis.

Resistance to Penicillin in Micro-organisms

With penicillin G clinically significant resistance is almost unknown except with the staphylococcus. Other organisms such as hæmolytic streptococci, pneumococci, meningococci and others have remained fully sensitive to penicillin G. In staphylococcal infections clinical failure is associated with the emergence of penicillinase-producing strains[59,60]. These, though capable of destroying large quantities of penicillin when once the enzyme has been induced, are inherently sensitive to it and may be destroyed by it if they cannot produce penicillinase quickly enough. On the other hand, in the laboratory it has proved easy to develop 'inherent' resistance of a non-penicillinase type in many different species of micro-organisms by serial sub-culture in gradually increasing concentrations of penicillin. It is uncertain whether a similar non-penicillinase type of resistance is likely to be of clinical significance in any infections except perhaps for the gonococcus[61]. It is also uncertain exactly how the penicillinase-producing types of staphylococci which are clinically so important actually arose. It is doubtful whether there is any proved case of the emergence of penicillinase-producing staphylococci of the same phage type from an originally sensitive strain during the treatment of an individual patient with penicillin. Thus penicillin shows the apparently paradoxical features of slow *development* of resistance and rapid *spread* of 'resistant', that is penicillinase-producing, strains by 'cross-infection'.

It will therefore be particularly interesting to see what happens with new penicillins. With methicillin a survey was carried out by Jevons at the Public Health Laboratory, Colindale[62]. Of 4,430 strains from different regions only one showed increased resistance to methicillin. So far it seems that methicillin-resistant mutants occur even more rarely than did penicillin G-resistant mutants in the early days of penicillin. They have not so far been found to destroy methicillin. In this respect they are rather more like the penicillin-resistant mutants produced by selection in the laboratory than naturally occurring penicillinase-producing strains. Experiments we have carried out in our laboratory[63,64] have shown that staphylococci resistant to methicillin, whether naturally or by artificial selection in the laboratory,

also show 'inherent' resistance to all other penicillins tested. But with a mutant naturally resistant to about 25 μgm./ml. of methicillin which showed 'inherent' resistance to penicillin G, further selection of mutants resistant to 1,000 μgm./ml. of methicillin was not accompanied by any further increase in resistance to penicillin G. More information is needed about the clinical importance and virulence of such mutants[45] and about cross-resistance in different penicillins.

So far we have considered penicillin resistance almost entirely in staphylococci, but the new 'Gram-negative' penicillin, α-aminobenzyl penicillin, will raise some new problems. Rolinson and Stevens[37] have pointed out that there is evidence for a penicillinase type of resistance in some strains of *E. coli*, *Proteus vulgaris* and *A. aerogenes*. It is clear now that each new penicillin as it is produced creates special problems of its own. Accurate information can be obtained only if careful records are collected in a number of different centres as a base-line for comparisons in the future.

Conclusion

The isolation of 6-aminopenicillanic acid has already led to the discovery of penicillins with new properties and has given a new impetus to antibiotic therapy. The search for new antibiotics has become something more than an empirical screening of many groups of organisms for some new kind of antibiotic activity. Deliberate and rational modifications to the penicillin molecule by the addition of specific side-chains have produced penicillins with different types of spectrum, and although the type of spectrum could not have been foretold from knowledge of molecular structure, once certain types of activity had been found, further modifications of the molecule could be made leading to improved activity of the kind desired. There still remain groups of organisms untouched by any of the new penicillins, for example, mycobacteria, pseudomonads and viruses. But the broad spectrum activity of a penicillin such as α-aminobenzyl penicillin raises some hopes that further modifications may produce other broad-spectrum penicillins, even perhaps some with antiviral activity. Quite apart from these practical results the isolation of 6-aminopenicillanic acid has opened up a fascinating field for research. The specific activity of different penicillins has raised many new questions about the so-called penicillin type of antibacterial spectrum and about the nature of penicillin resistance; and at the same time it has helped to provide some of the answers, which may be of great value in relation not only to penicillin and other antibacterial drugs but also to the specificity of enzymes and their biosynthesis. The diagram (Fig. 2) represents an attempt to put into concrete form some of the conclusions which logically follow from the facts known at the present time. It must inevitably be inaccurate in part and may even be regarded as a caricature of the facts. If it does nothing more than arouse constructive criticism it will have served its purpose.

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TRANSFER OF STRATOSPHERIC FISSION PRODUCTS INTO THE TROPOSPHERE

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MOST programmes of fall-out measurement are designed primarily to provide a regular inventory of the radioactivity from nuclear test explosions with the purpose of computing the radiological dose to human populations. In addition, the radioactive debris during its passage through the atmosphere serves as a valuable tracer of meteorological processes.

The significant parameters in this meteorological tracer experiment fall into two classes. Measurements are made of the concentration in air or in rain-water of (*a*) the long-lived fission products, as an index of the rate of fall-out, and of (*b*) the ratio of short-lived to long-lived fission products to give the 'age' of the debris, that is, the ratio of mixing between debris from recent and earlier test explosions. The mixing ratio is derived from comparison of the measured fission product ratio with that calculated for a fresh mixture. The data for several of these tracers is assembled in Table 1, using the yields from fission by 14-MeV. neutrons of uranium-238 published by Cunninghame[1] and by Ford and Gilmore[2].

Table 1. FISSION PRODUCT DATA

Fission product	Half-life	Fission product ratio	Value of ratio at date of fission
Cæsium-137	30 years	Sr^{89}/Sr^{90}	185
Strontium-90	28 years	Zr^{95}/Cs^{137}	145
Cerium-144	285 days	Ce^{144}/Cs^{137}	~ 28
Zirconium-95	65 days		
Strontium-89	50 days		

The present discussion is concerned with the mixing between fission products from the 'polar' stratospheric injections at 75° N. in October 1958 and those from earlier injections, in particular from 'equatorial' injections at 11° N. about June 1958. Evidence relevant to the transfer of fission products from stratosphere to troposphere will be presented in three forms, describing the variation of the fall-out parameters with latitude, time and with altitude. This evidence is based on measurements made at Harwell, which are in substantial agreement with the results of work reported elsewhere, notably in the United States by Lockhart *et al.*[3,4].

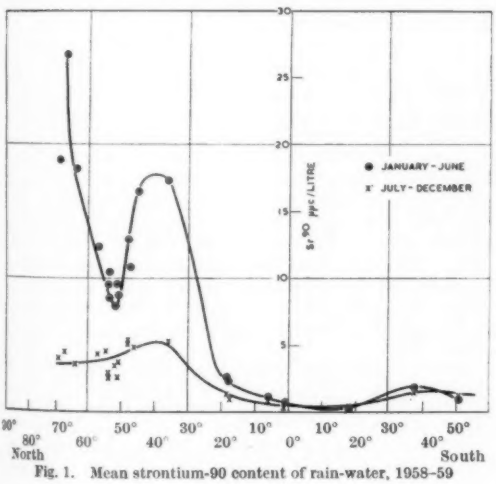

Fig. 1. Mean strontium-90 content of rain-water, 1958–59

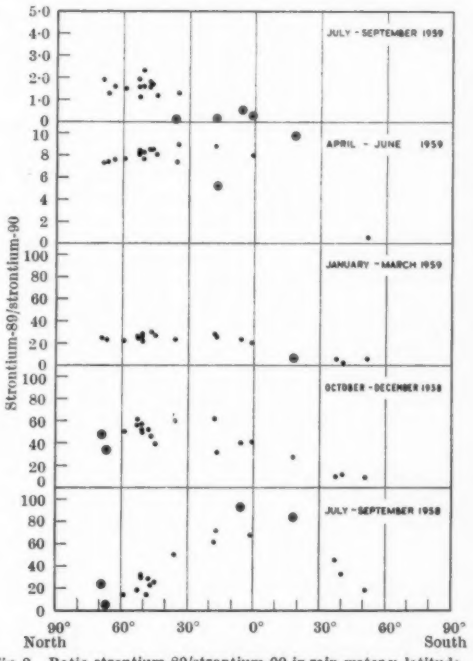

Fig. 2. Ratio strontium-89/strontium-90 in rain-water v. latitude, 1958–59

[○, Uncertain ratio due to low activities]

Variation with Latitude

The variation with latitude of the strontium-90 content of rain-water has been published by Crooks *et al.*[5] for the period 1957–59. The main features of the variation, averaged over this period, is the rise to maximum values in the northern hemisphere in sub-tropical latitudes and above 60° N. The relative distribution varies with season as in Fig. 1, which shows the mean concentrations of strontium-90 during the months January–June and July–December. Although the equatorial minimum is maintained throughout the year, the distribution above 30° N. is comparatively uniform during the second half of the year.

A qualitative impression of the origin of the fission products during 1958–59 may be obtained from a study of the ratio strontium-89–strontium-90 in the same rain-water samples[6]. The variation of this ratio with latitude is shown in Fig. 2 for three-monthly periods from July 1958 to September 1959.

From October 1958 the ratio strontium-89 – strontium-90, and hence the ratio of polar to equatorial debris, remained substantially independent of latitude in the northern hemisphere. In the period July–September 1958, however, stratospheric fall-out from injections of fission products near the equator increased the ratio strontium-89–strontium-90 at low latitudes in both hemispheres.

The content of strontium-89 in samples collected during this period is assigned to a stratospheric source because of the behaviour of the ratio[6] strontium-89–strontium-90 with time. In the southern hemisphere, the ratio decreases from June 1958 according to radioactive decay. The same observation can probably be applied to samples collected in the northern hemisphere, although in this case the record is confused by the arrival of fresh radioactivity from the test explosions of October 1958. If the strontium-89 were primarily tropospheric, it would disappear more rapidly, at a rate consistent with a tropospheric residence-time of 30 days[7].

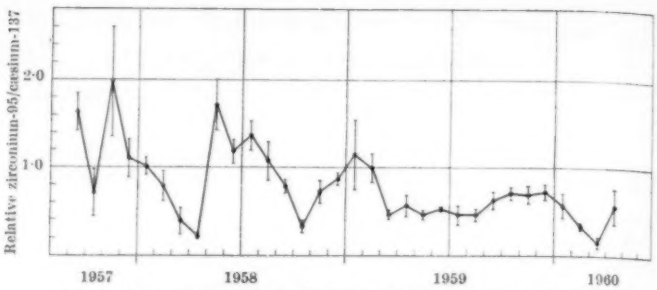

Fig. 4. Ratio zirconium-95/cæsium-137 at 47,000 ft. relative to 4,000 ft.

Variation with Time

A periodic variation in fission product concentrations has been demonstrated[5,8] for samples taken from the troposphere and from rain-water in temperate latitudes of the northern hemisphere. It was suggested by Stewart *et al.*[9] that the observed periodicity is consistent with the meridional circulation in the stratosphere postulated by Brewer[10] to explain the low water content of the temperate stratosphere. Recently, Goldsmith and Brown[11] have suggested that this circulation is confined to the lower stratosphere. Martell[12] attributed the seasonal increase mainly to rapid fall-out from Russian stratospheric injections at high latitudes in the immediately preceding autumn and winter. However, the spring maxima in 1955, 1960 and in 1961 followed autumns without stratospheric injection, and it can be shown that a peak remains in 1959 after the effect of stratospheric injections during the previous autumn has been subtracted[8]. It is therefore probable that the seasonal cycle has a meteorological origin.

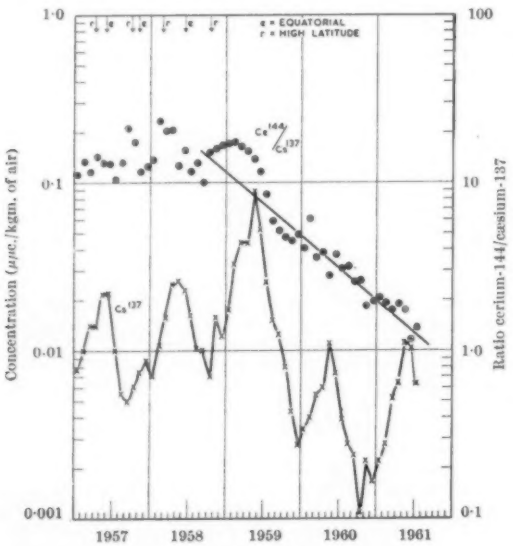

Fig. 3. Concentration of cæsium-137 and cerium-144 in air near ground-level at Chilton, Berkshire

In considering the variation with time of the isotope ratios, it has already been noted[8] that at ~55° N. the ratio zirconium-95–cæsium-137, in the case of tropospheric samples, and the ratio strontium-89–strontium-90 (rain-water samples) decreased according to radioactive decay, indicating uniform mixing of new and old debris, during the first six months of 1959. About June 1959 a discontinuity occurred in the isotope-ratio curves. This effect is repeated in the record[6] of cerium-144–cæsium-137, shown in Fig. 3, which extends the observations in air near ground-level at Chilton (Berkshire) until mid-1961. Owing to uncertainty in the fission yield data, there is not exact agreement in the proportions of polar and equatorial debris calculated from the various isotope ratios. Nevertheless, using the cerium-144–cæsium-137 results, it is clear that whereas during January–June 1959 the proportion in these samples of long-lived debris produced by the Russian polar injections of October 1958 was about 70 per cent, for the period from mid-1959 until mid-1961 this proportion decreased to a value not greater than 20 per cent. This lower proportion was maintained through the subsequent spring peaks in 1960 and 1961, although the individual fission product activity varied by a factor of about five between spring and autumn.

Variation with Altitude

This evidence is less general than the previous forms, in that the observations at the upper altitudes are restricted to the vicinity of the United Kingdom. In January and February 1959 the ratio of polar to equatorial debris (measured by zirconium-95–cæsium-137) in the lower stratosphere at 47,000 ft. and ~55° N. was similar[8] to that observed later in the troposphere. During the remainder of the year (Fig. 4), the ratio zirconium-95–cæsium-137 was significantly higher in the troposphere than in the lower stratosphere[8], implying a higher proportion of polar debris in the troposphere.

A Model of the Transfer Mechanism

The observations summarized above provide, in combination, a set of conditions that must be satisfied by any model of the transfer between stratosphere and troposphere. One possible model will be described

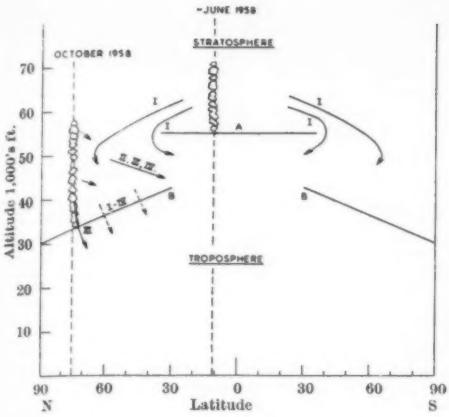

Fig. 5. Transfer from stratosphere to troposphere

A, equatorial tropopause
B, temperate-polar tropopause
I, July–September 1958
II, October–December 1958
III, January–March 1959
IV, April–June 1959

that attempts to explain the behaviour of the debris from the stratospheric injections in 1958 ('Hardtack') at 11° N. about June and Russian at 75° N. during October. Because the measurements of fission products are reduced to monthly average values, the model cannot account for short-term meteorological changes.

The movement of the debris is illustrated in Fig. 5, which is a schematic diagram of the atmosphere showing stratosphere and troposphere separated by nominal tropopauses, equatorial and temperate-polar. Stratospheric fission products from the equatorial injection passed readily through the gap between equatorial and temperate tropopauses (Period I of Fig. 5). On the other hand, the polar debris appeared in the troposphere only after reasonably complete mixing with older debris: the mode of entry during the autumn of 1958 (II) appears to be mainly through the same gap, but also possibly through the temperate-polar tropopause.

It is only during the early months of 1959 (III) that a significant route appeared at high latitudes in the temperate-polar tropopause, in temporary competition with the gap between equatorial and temperate tropopauses. Since the area of a band of latitude decreases with increasing latitude, it should be noted that the high concentrations occurring polewards of 60° N. refer to only a small proportion of the total activity of the fission product. The timing of this phenomenon is in accordance with the Brewer–Dobson circulation, in which the lower stratosphere is supplied in mid-winter by a subsidence of cold polar air. Despite the two possible paths of entry during this period, tropospheric samples were of such uniformity in the ratio of fission products (Fig. 2) that the two streams must have arisen from a well-mixed source of equatorial and polar debris. The mixing of debris must have occurred either in the lower stratosphere or in the troposphere. If mixing occurred in the troposphere, under the influence of a tropospheric circulation system, then it is difficult to reconcile the uniform distribution of the ratio strontium-89–strontium-90 with the non-uniform distribution of strontium-90 (Figs. 1 and 2). It is necessary, therefore, to postulate that mixing took place in the lower stratosphere to account for uniformity of the ratio of fission products, and that the distribution of individual fission products with latitude is probably controlled by the points of entry through the tropopauses, assisted by the tropospheric circulation system.

In spring and early summer 1959 (IV), the exchange through the temperate-polar tropopause diminished, leaving the gap between equatorial and temperate tropopauses as the dominant path of entry. By this time the source of polar debris was largely exhausted and the supply to the troposphere contained a higher proportion of equatorial fission products.

This new mixing ratio was maintained from mid-1959 through 1960 and into 1961 (Fig. 3), apparently undisturbed by the Brewer–Dobson subsidence that would occur in the winters of these years. This more recent evidence suggests that any residual polar debris (October 1958) was stored higher in the stratosphere with equatorial debris from 'Hardtack' (1958), and probably from earlier test explosions such as 'Redwing' (1956) and even 'Castle' (1954). The polar debris appeared as a small fraction of the well-mixed tropospheric samples collected during 1960 and 1961. This fraction, calculated from the ratio cerium-144–cæsium-137, is consistent with a date of origin lying between November 1957 and February 1958. A similar calculation made by Parker and Crookall[13] for samples collected during 1960 gave a mean date of origin in April–May 1958. The two sets of experimental ratios of fission products are in good agreement: the difference in estimated date is due to the assumption of different fission yields for the two isotopes.

It was observed that during most of 1959 the proportion of polar debris sampled in the lower stratosphere over the United Kingdom was lower than in the troposphere. It is difficult to fit this evidence into the scheme proposed in Fig. 5. Since it may represent a local anomaly, it is sufficient at present to note that in the latitudes of the United Kingdom the stratosphere and the troposphere maintained separate identities during this period. This suggests that the two regions of the atmosphere are supplied from reservoirs of radioactive debris by separate routes and that there is little local mixing between them. Alternatively, the difference in isotope ratio could be explained by a wash-out mechanism in the troposphere that favoured selective removal of older debris, or by selective filtering in the air-sampling apparatus. From other evidence both these explanations are unlikely.

Conclusion

The variations of certain fall-out parameters, namely, the concentration and ratio of fission products, have been summarized in relation to latitude, time and altitude. These phenomena are influenced by the mechanism of transfer of fission products from the stratosphere into the troposphere.

A speculative model of this mechanism has the main features that follow, based upon the behaviour of fission products injected into the stratosphere in the summer and autumn of 1958.

Debris from the polar injection was well mixed with other debris before entry to the troposphere. The tropospheric circulation system may contribute to the distribution pattern of the debris with latitude, although this appears to be controlled by the points of entry through the tropopauses.

Well-mixed debris escaped in the early months of 1959 through the temperate-polar tropopause at

latitudes greater than 60° N. This escape was probably associated with subsidence of stratospheric air, according to Brewer and Dobson, and suggests a virtual disappearance of the troposphere at these latitudes.

Most of the polar debris appeared in the troposphere during the first six months of 1959. A small residue from the polar injection may have lodged above the lower stratosphere. This residue would have been brought down, together with older debris, during subsequent winter subsidences, and appeared as a well-mixed fraction of the fission product concentrations measured during 1960 and 1961.

The proportion of polar debris in the lower stratosphere above the United Kingdom in 1959 was lower than in the troposphere. This effect may be restricted in extent and have no great significance in a global model.

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ELECTRICAL POWER DEVELOPMENT

THE annual meeting in June of the British Electrical Power Convention was held, as has been customary in recent years, at Eastbourne, under the presidency of Sir John Pickles, chairman of the South of Scotland Electricity Board. In his presidential address he gave emphasis to the theme of the Convention—"Electricity in the Prosperity and Welfare of the Nation". The address was in the form of a review and prospect of the electricity supply industry in Britain from A.D. 1880 to 2000.

Sir John considered that there would be a doubling of consumption in the ten years to 1970 and that this rate of increase would be maintained, with a further doubling by 1980, or 1985 at the latest. This implies an increase in the country's installed generating plant capacity, at present 32,000 MW., up to 100,000 MW., the greater part of this probably being nuclear. There would probably be 1000-MW. single generating sets, and their operation, together with that of their boilers, would be controlled by computers. In transmission a 750-kV. system seems not unlikely. Sir John concluded by referring to the possibilities of direct conversion of heat to electricity without the intervention of a steam cycle, attention being focused on thermionic, thermo-electric and magnetohydrodynamic generation. The latter, comprising direct generation by the passage of hot gas through a magnetic field, is the most promising. Nuclear fission may well prove highly suitable as a heat source.

The second speaker, Sir Christopher Hinton, chairman of the Central Electricity Generating Board, described the British electricity distribution system from the inception of the 132-kV. grid, which was intended for the transfer of energy between the large self-contained areas under conditions of difficulty or emergency. Much expert opinion at that time held that it would be impossible to interconnect the complete system without leading, under conditions of rapidly changing demand, to major power flows which could not safely be handled. Notwithstanding this, the London control engineers interconnected all the separate areas by means of the grid "to see what would happen". The interconnected system—the largest in the world controlled from one centre—has worked ever since. Various extensions and reinforcing interconnexions have been added, giving additional security and flexibility.

Work carried out at the formation of the British Electricity Authority and the area boards under the Electricity Act of 1947 showed a sound case for siting new generating plant near coalfields. This implied the provision of a higher voltage transmission system superimposed on the existing grid. A transmission voltage of 275 kV. was selected, this being the next higher International Electrotechnical Commission standard. It was considered that this voltage is not so high as to lead to possible insulation difficulties in the atmosphere of Great Britain. Tower clearances, however, allow for up-rating in the future to 380 kV.—the next higher level—when insulation problems have been solved. So the 275-kV. super grid system in Britain was formed, developing mainly into ring mains of double-circuit lines connecting large-capacity generating stations and substations with transformation, usually to 132 kV. Transmission is by no means the junior partner of generation: it is perhaps dominant, for without the present highly developed transmission system—planned with foresight and wisdom by the old Central Electricity Board and those associated with it—the modern 2000-MW. generating station would be impracticable.

Sir Christopher Hinton reported that the maximum demand on the system so far was 24,449 MW., on December 13, 1960, and he suggested that this might be trebled in fifteen to twenty years time. The heaviest concentration of power stations would be in the central coalfields, with others in North Wales, the Severn Estuary, the Bristol Channel, the central south coast, the Thames Estuary, south-east Kent and the East Anglian coasts. He showed a map giving the distribution of power expected in the mid-1970's, when the maximum demand is likely to be 70,000 MW. and the projected 400-kV. supergrid fully operative. Nuclear generation should by then account for 30 per cent of the total consumption, indigenous fuel and oil consumption being roughly doubled. The movement of coal long distances by rail should virtually have ceased by this time, and sea-borne supplies reduced to roughly half their present value. As the load grows beyond 70,000 MW. in the years after 1975, it is possible that a 700-kV. 'large mesh' network will be superimposed on the 400/132-kV. system.

Sir Christopher continued by explaining the many factors not susceptible of control by the area boards which make accurate forecasting of future needs very difficult. He referred to shifts of population, town planning legislation and Government decisions on residential and industrial development. Forecasting is simple on a national scale and difficult on a local scale.

About 40 per cent of the area of England and Wales is subject to restriction of some sort or other on the ground of amenity ; but opposition to the construction of transmission lines and equipment is sometimes just as severe in industrial towns. Yet a transmission system of the present type is vital to British industry and essential if the very large stations (small in number and of high efficiency) are to be built. Although the public would not tolerate the ever-increasing extension of the network to a stage rightly described as a 'wirescape', underground cables for all additions to the network are out of the question on ground of cost. (A figure of about £100 million a year would be involved, representing 6*d*. on the standard rate of income tax, or 20 per cent on the bulk tariff.) The solution, perhaps a compromise, is to place underground the lower-cost distribution systems and leave the very high-voltage transmission system in the form of lines on towers. The latter must be accepted as canals, railways and motorways are, but the design of the structures must be the most graceful that can be devised and their routing must be most carefully planned. Sir William Holford set out in a paper to the Royal Society of Arts valuable rules relating to the siting of transmission lines. Switching stations, too, are now being most carefully considered. Sir Christopher Hinton concluded by emphasizing that the problem of visual amenity is one of the greatest facing the industry.

Mr. R. R. B. Brown, chairman of the Southern Electricity Board, presented a paper entitled "Some Aspects of Efficiency and Economy in Distribution". He reported that the national investment in distribution assets stands at more than £1,000 million, and additional expenditure at the rate of about £100 million annually is necessary to meet the expanding needs of the nation. Mr. Brown showed ways in which investment economies have been and will be achieved ; and he dealt with tariff structures devised to encourage the full and efficient use of distribution systems. An interesting point made in connexion with standardization was that less than a hundred thousand consumers out of some 16 million now receive direct current supplies ; consumers served by non-standard alternating current systems number about 1,500,000.

A paper by Dr. A. E. Grauer, chairman and president of the British Columbia Electric Co., Ltd., was on "The Electrical Power Industry in Canada". Canada, with a population of only 18 millions, is one of the world's largest producers (and consumers) of electric power. On a total output basis, she is behind the United States and the U.S.S.R., but on a par with the United Kingdom. Canada is second only to Norway on a *per capita* basis. Much of Canada's power is, of course, hydro-electric, and in Quebec power-rates are less than a third of the Canadian-American average. In Ontario and Manitoba they are about half the average in the United States. Canada's impressive power growth has been predominantly due to important power-consuming industries, smelting aluminium, refining copper, nickel and titanium and manufacturing inorganic chemicals. This growth has helped to open up new territory and expand Canada's world trade.

It is interesting that primary jurisdiction over natural resources, including water, was given to the Provincial Governments rather than to the Federal Government, and this, together with the huge areas of most of the provinces, has resulted in electrical utilities each essentially operating within one province. There are no electrical utilities which are national in scope.

Much of Canada's land mass (in area almost equal to China, which is itself second only to the U.S.S.R.) is virtually uninhabited, and population has tended to concentrate in a few areas strung mostly along the southern border and separated from each other by hundreds of miles. Almost a third of the population lives in the southern tip of Ontario. Nearly ten million Canadians occupy an area less than that of the United Kingdom.

The manner in which the electric power industry is organized varies a great deal from one province to another. In some provinces there is practically no private ownership, in others practically no public ownership ; in others there is a mixture of both. The sizes of the utilities differ considerably, from Ontario Hydro, with a total capacity in excess of 6,000 MW. to quite small undertakings. Publicly owned utilities are usually run by a small commission reporting directly to the Cabinet. Investor-owned utilities operate under a Public Utilities Act administered by a Commission appointed by the Provincial Government. Fair rates are set by the Commission.

Canadians used, in 1959, 4,300 units of electrical energy per residential customer as compared with 3,700 units in the United States and about 2,000 units in the United Kingdom. One possible reason is that about 80 per cent of Canadians live in single or duplex dwellings. Also, the straight block-rate, with no demand charge, is almost universal, except for house heating. Off-peak tariffs are not used in Canada : industry works mainly on a three-shift system. It is interesting that Dr. Grauer's Company estimates each customer's bill for a ten-month period, sends him a bill each month for a tenth of this amount, and then balances the books by a payment of the difference either way at the end of the period.

Regarding rate of growth of electrical consumption, a relatively low figure might be expected from an already high consumption. Estimates point to an average figure of more than 6 per cent per annum and require the construction of more than 50,000 MW. of new capacity by 1980. It is expected that most of the money to finance this will continue to be raised in Canada. This does not necessarily apply to the Peace River (British Columbia) and Hamilton Falls (Labrador) developments. It would appear that here are opportunities for British investment in Canada.

Canada's commercial water-power potential is considered by Dr. Grauer to be 70,000–80,000 MW. : the present installed turbine capacity is about 20,000 MW. The largest undeveloped hydro resources are in Quebec and British Columbia, with Newfoundland and Manitoba following. Canada and the United States have, of course, signed a treaty respecting the Columbia River and construction is expected to start in 1962, with output of electrical energy by 1966.

Dr. Grauer made some interesting remarks concerning the development of thermal generation. The latter is necessary in some main industrial

centres because of the existing full development of nearby water resources and because of the increasing costs of more remote hydro development. Steam-electric generation near the load centres is tending to cost less, improvements in design offsetting inflation. The margin of cost between hydro and thermal generation is thus narrowing steadily. A large coal-burning base load plant on the waterfront of Toronto can deliver electric power at a cost only about fifty per cent higher than can the new hydro-installations at Niagara and on the St. Lawrence Seaway. (About 1920 thermal electric power cost three to five times as much as hydro-electric power in southern Ontario.) Provinces using thermal generation in the future will be Alberta, Saskatchewan (with cheap fossil fuels and natural gas), Ontario (with almost fully developed hydro-resources she is importing coal from the United States) and British Columbia (with cheap residual oil and natural gas). Gas turbine plants are being built in sizes from 2,000 to 25,000 kW., but are unlikely to be used extensively because of their heavy fuel consumption compared with that of large steam turbines.

Canada has several large research reactors in operation, being particularly interested in heavy-water moderated systems fuelled by natural uranium. (Heavy water has been produced in Canada and could be again. Uranium is found in abundance.) The large conventional resources still available in Canada make it likely that nuclear power will first be commercially developed elsewhere, with southern Ontario as an exception to that statement in view of its present need to import coal from the United States.

Dr. Grauer made some useful comments on long-distance transmission and the possibilities of a national power grid. Most transmission in Canada is at 230 kV., with 360 kV. being introduced in recent years. Distances of transmission have not exceeded about 250 miles, but much longer distances are in prospect. Ontario Hydro is planning 500 miles of 460-kV. line. The Peace River proposal envisages 600 miles at 500-600 kV., and tests are under way, both in Canada and the United States. Present thinking in the U.S.S.R. is that distances less than 600-800 miles are more efficiently met with alternating current, while for distances greater than this, direct current is more efficient. Canada's distances are similar to those in the U.S.S.R. However, potential developments are so placed relative to population centres that few distances of greater than 600 miles are contemplated. Three large potential hydro-situations in the far north are about a thousand miles from the chief market and their development will presumably await the d.c. transmission of the future. Dr. Grauer believes that a national power grid, for a number of reasons (not the least being that natural gas in large pipe-lines can be transported for less than one-third the cost of transporting electrical energy over the highest voltage lines) is more likely to evolve over a period of time from regional systems stronger and more highly developed than exist to-day.

The final paper of the conference, presented by Mr. S. F. Steward, director of the British Electrical and Allied Manufacturers' Association, was entitled "British Electrical Manufacture in the National Economy". The British Electrical and Allied Manufacturers' Association, the trade association of the electrical industry in Britain, is this year celebrating the fiftieth anniversary of its foundation. The main aim in establishing the new trade association in 1911 was "to foster some order and regulation in the trading of its constituent firms". It is interesting to note that within a year or two of the Association's foundation the annual report invited "the earnest attention of members to industrial and scientific research as one of the bedrocks of commercial supremacy".

Early attention was given to the legal conditions applicable to contracts for heavy plant, and, in association with the Institution of Electrical Engineers, model general conditions were drawn up which, when published in 1914, became the first standard text of its kind in the world. Technical and commercial co-operation, publicity and export promotion were early recognized as main functions of a trade association. Standards were issued as early as 1913 for the use of member firms, priority being given to the standardization of electrical machinery.

Mr. Steward reviewed in considerable detail the development of the electrical industry throughout five decades, emphasizing the extent to which electrical manufacture has become a key industry. About ten per cent of the total United Kingdom export of manufactured goods represents electrical equipment. The total number of employees in the industry is nearly 800,000, 28 per cent of this number being engaged in the manufacture of electrical machinery and 38 per cent on electronics and telecommunications equipment. Electrical manufacture is the largest industrial employer of scientists and technicians. In 1959 the industry employed 13,380 qualified scientists and engineers.

The drawing up of conditions of contract and contributions towards the establishment and maintenance of standards continue to be important activities of the Association. Of increasing importance is the collection and dissemination of statistics and information, the study of trends and of overseas competition. "A trade association must be a realistic partnership of competing concerns, not with the purpose of restricting competition but to secure the best use of an industry's resources and to provide the basis for the full development of initiative and enterprise".

A. J. Ellison

OBITUARIES

Prof. M. L. Anderson

The death of Prof. M. L. Anderson, of the chair of forestry in the University of Edinburgh, occurred on September 6 at Bristol, while he and Mrs. Anderson were on a visit to their married daughter.

Mark Louden Anderson was born on April 16, 1895, at Kinneff in Kincardineshire. He had the misfortune to lose both parents when he was only eleven years old. He entered the forestry course at the University of Edinburgh in 1912, but saw service throughout the First World War, first in the Argyll and Sutherland Highlanders and the Black Watch, and then in the Machine Gun Corps, winning the Military Cross in 1918. Returning to Edinburgh, he graduated B.Sc. (Forestry) in 1919, and five years

later was awarded the D.Sc. degree for a thesis on tree form and volume studies.

Prof. Anderson was appointed to the Forestry Commission and as a research officer he had much to do with early experiments in planting on peat, provenance of species trials and the selection and measurement of sample plots. His work was conducted with that depth of vision so necessary in practical research. It was unfortunate that he had to give up this particular work when he did, but Eire gained his services and there he eventually became director of forestry. This was followed by five years on the staff of the Department of Forestry, University of Oxford. It was then his intention to take up a post offered by the Australian Forest Service, but instead he returned to the University of Edinburgh to be professor of forestry, a post which he occupied with distinction for ten years.

Prof. Anderson was an enthusiastic forester who showed his keenness in a variety of ways. He enjoyed being in the forest, and he regretted when ill-health prevented him from visiting again the Continental forests, where he admired the practice of good sylviculture and sound management. At any time he would discuss forestry matters and even provoke controversy for the fun of enjoying the outcome. As a teacher, Prof. Anderson was never verbose. His great knowledge was readily available to the keen forester, no matter what the latter's rank was. He believed that a good forester should have a proper basic knowledge and know how to observe. He had a wonderful gift for languages, most of which were self-taught, and he tried by his numerous translations from French, German, Danish, Norwegian, Swedish, Finnish and Russian books and papers to bring important works of Continental foresters to the English-speaking world. Knuchel's *Planning and Control in the Managed Forest*, Kostler's *Silviculture*, Larsen's *Tree Genetics*, Petrini's *Forest Economics* and Tamm's *Northern Coniferous Forest Soils* are some of the larger translations. Prof. Anderson's *The Selection of Tree Species* is a very valuable guide for the British forester and it is now in its second edition. Not long before he died, Prof. Anderson completed the manuscript of his monumental work, *A History of Scottish Forestry*—the product of some thirty years of painstaking research. It is to be hoped that financial difficulties will be overcome to allow this encyclopædic work to be published.

Another aspect of Prof. Anderson's keenness to stimulate an interest in forestry and to try to raise its general standard was the zeal and time he gave to forestry societies. He was an ardent member of the Royal Scottish Forestry Society and recently its honorary secretary and the creator of its Silvicultural Group, a founder member of the Society of Foresters of Great Britain and also of the Forestry Society in Eire. He was also a regular contributor to *Nature*.

But forestry did not occupy all Prof. Anderson's very full life. He found great delight in transcribing old Scots records, and in this he took infinite care to obtain accurate results from folios which, at times, were rather difficult to decipher. As a result of this work he was a regular contributor to the *Dictionary of the Older Scottish Tongue*. His *Proverbs in Scots* were published in 1957, another example of painstaking work on a manuscript dating from the first quarter of the seventeenth century.

Prof. Anderson will be remembered as a great forester, an ardent Scot, a man respected for his integrity, his capacity for hard work, his willingness to help others, his friendliness and his great determination.

Prof. Anderson leaves a wife, two sons and a daughter, to whom our sympathy is extended.

C. J. Taylor

Dr. W. A. MacFarlane, C.B.E.

The sudden death on August 19 of Dr. W. A. MacFarlane, managing director of the National Industrial Fuel Efficiency Service and president of the Institute of Fuel, at the age of fifty-three, came as a severe shock to his many friends and is a grievous loss to fuel technology in Great Britain.

Although Angus MacFarlane was by training, and in his early work, a 'pure' chemist, the major part of his varied life was concerned with the efficient use of fuel. After a brilliant career at Oxford, where he was a student of Sir Harold Hartley at Balliol, he spent two years at the University of California as a Commonwealth Fund Fellow carrying out research in physical chemistry for which he was awarded the degree of Ph.D.

On his return to Britain in 1934, after eight years in academic life, he joined the staff of the Fuel Research Station of the Department of Scientific and Industrial Research and thus first came into contact with fuel technology. During the four years at East Greenwich his main contribution was the initiation of the Department's work on the Fischer–Tropsch process, which was destined to become one of the major research programmes of the Fuel Research Board, and to continue for a total period of some twenty-five years. In 1938 MacFarlane left the Fuel Research Station to become senior research chemist to the London, Midland and Scottish Railway Co. at the invitation of Sir Harold Hartley, then vice-president of the Company. This move naturally led him into wider fields, but he was soon to return to fuel, for in 1941, after the formation of the Ministry of Fuel and Power, he was appointed the first director of fuel efficiency. In this position he made an important contribution to the War effort by ensuring that every ton of coal was directed where it was most needed and used to the best advantage.

After seven years at the Ministry, MacFarlane was appointed to the British Embassy at Washington as director of the United Kingdom Scientific Mission and attaché for scientific questions. During his six years in Washington he did a great deal to foster and cement Anglo-American friendship and co-operation in the scientific sphere and there must be very many who, like myself, remember with gratitude the efficient help and warm hospitality he always extended to British scientists visiting the United States.

In view of his earlier work at the Ministry, the selection of MacFarlane as director of the National Industrial Fuel Efficiency Service on its formation in 1954 was an obvious one, and the undoubted success of this organization in conserving fuel and reducing costs to the fuel user offers testimony to his efficient management. His services to the nation were recognized when he was made a C.B.E. in 1958, and his election to the presidency of the Institute of Fuel in 1960 is evidence of the high esteem in which he was held by his fellow fuel technologists.

MacFarlane combined a sound scientific knowledge with an ability to appreciate clearly and rapidly the practical and economic aspects of a problem. He had the gift of inspiring loyalty and affection in those

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working under him, and his intellectual gifts and genial personality won for him a very wide circle of friends by whom he will be sadly missed.

He leaves a widow and four children.

C. C. Hall

Mr. H. V. Griffiths, M.B.E.

The sudden death of H. V. Griffiths on June 28, at the age of fifty-nine, came as a shock to his many friends and colleagues.

As engineer-in-charge of the British Broadcasting Corporation's Receiving and Measuring Station at Tatsfield, he was widely known both in Britain and overseas as an authority on radio reception, propagation and frequency measurement. He represented the B.B.C. on a number of technical committees concerned with these subjects, about which he has written from time to time in *Nature* and other journals.

Like many of his colleagues in the early days of radio, Griffiths started his career as a ship's radio operator, but his interest was in the technical, rather than the operational, aspects of his work, and it was not long before he entered the engineering field. He joined the B.B.C. in 1924 as an assistant maintenance engineer and later became a member of a team of engineers to work on the first short-wave broadcast transmitter used at Chelmsford by the B.B.C. He also co-operated with Marconi engineers in the study of diversity reception on short waves.

He was appointed engineer-in-charge at the Tatsfield Receiving and Measuring Station in 1933 and over the years he built up for Tatsfield a reputation for accuracy and reliability which has made the Station a world-wide name in radio. His work was recognized when he was made M.B.E. in 1958.

Among his many other interests was astronomy. He was a Fellow of the Royal Astronomical Society and was keenly interested in man-made satellites and their future possibilities in the field of world-wide radio communication. He took an active part in monitoring the transmissions from the first *Sputnik*.

H. V. Griffiths will be greatly missed by his many friends and colleagues, who will remember him not only for his expert knowledge, which he was always ready to pass on to others, but also for his sincerity and kindly patience and, above all, his enthusiasm for his work.

L. W. Turner

NEWS and VIEWS

Iodine-131 in Milk in Britain

The first results of an investigation of iodine-131 in milk, which has been in progress at the Agricultural Research Council Radiobiological Laboratory since weapons tests were resumed by the U.S.S.R., were released on October 24 simultaneously with a general statement by the Medical Research Council (see *Nature*, November 4, p. 400). A further statement from the Agricultural Research Council (dated November 1) summarizes more recent measurements. Milk from twenty depots in central and southern England, Wales, Scotland and Northern Ireland is now being collected daily and examined for levels of iodine-131. This represents a considerable expansion of the sampling which has been carried out during the past six weeks at eight sites in different regions (see Table 1). These measurements enable the general trend throughout the United Kingdom to be assessed.

Table 1. Levels of Iodine-131 in Milk produced at Eight Sites in Different Parts of the United Kingdom

Week commencing:	μμc. Iodine-131/litre
September 16, 1961	85
September 24, 1961	109
October 1, 1961	138
October 8, 1961	150
October 15, 1961	114
October 22, 1961	158
Mean for six weeks (September 16–October 28)	126

Information on regional differences of iodine-131 is now obtained each week by analysing samples collected from more than 200 depots which handle more than 40 per cent of the total milk produced throughout the United Kingdom. The samples are combined in proportion to the quantity of milk handled by the depots from which they are obtained so that the average level throughout a zone can be ascertained. The present results are summarized in Table 2.

Table 2. Levels of Iodine-131 in Milk produced throughout the United Kingdom

Mean results for samples collected on October 2, 16 and 23, 1961

Zone	μμc. Iodine-131/litre
1. Northern England	111
2. North-West England	100
3. East and East Midland	82
4. West Midland	106
5. Wales	174
6. South and South-East England	99
7. Mid-Western England	96
8. Far Western England	143
9. Scotland	178
10. Northern Ireland	161
Country-wide mean weighted for production	121

The country-wide mean of 126 μμc. per litre for the six weeks ending October 28, which is shown in Table 1, represents about one-eighth of the total quantity of iodine-131 which would give rise to the annual average of 130 μμc. per litre specified by the Medical Research Council. The results in Table 2 show that, on a regional as well as a national basis, the iodine-131 which has hitherto entered milk represents but a small fraction of that which would give rise to the level specified by the Medical Research Council. Further statements will be released at intervals until the levels of iodine-131 in milk have decreased appreciably.

Theoretical Physics in the University of Sussex: Prof. R. J. Blin-Stoyle

Dr. R. J. Blin-Stoyle has been appointed to the chair of theoretical physics in the new University of Sussex. He attended Alderman Newton's Boys' School in Leicester, and received a senior scholarship at Wadham College, Oxford, in 1943, but before taking this up in 1946 was commissioned into the Royal Signals and served as lecturer in the College of Radio Communications at Mhow, Central India. He received a B.A. (1949), D.Phil. (1951) and, during 1951–53, held a Pressed Steel Fellowship at Oxford. After a year as lecturer in mathematical physics in

the University of Birmingham he took up his present post at Oxford as senior research officer in physics. Since 1956 he has also been a Fellow and lecturer at Wadham College. During 1959–60 he served as visiting professor of physics at the Massachusetts Institute of Technology and the University of California.

Prof. Blin-Stoyle is widely recognized as one of the ablest young theoretical nuclear physicists. He has made valuable contributions to the theory of nuclear structure, with special emphasis on nuclear moments and β-decay. His main object in this work is to use the nuclear properties to learn about more fundamental things, such as the possible charge dependence or parity non-conservation of nuclear forces, and the nature of weak interactions. He has also been concerned with the theory of quantum fields and reactions of polarized nuclei. He is much appreciated as a tutor and lecturer as well as for his research work and, although his departure will be sadly felt in Oxford, he should have greater possibilities at Sussex for implementation of his progressive ideas on the teaching of physicists. His appointment promises much for the future development of physics at the new university.

Engineering Group of the United Kingdom Atomic Energy Authority :

Mr. J. B. W. Cunningham

Mr. James Bernard William Cunningham, who resigns as managing director of the Engineering Group of the U.K. Atomic Energy Authority as from January 20, 1962, was born in Middlesbrough, Yorks., on December 9, 1911. During 1928–39 he served as an apprentice with Cochrane and Co., Ltd., Middlesbrough, and Stanton Ironworks Co., Ltd., Middlesbrough, rising to works manager. During 1939–44 he was assistant works manager at Dewrance and Co., Ltd., London, and during 1944–47 was works manager at Pegsons, Ltd., Coalville. Mr. Cunningham then joined the Department of Atomic Energy of the Ministry of Supply at Risley, Lancs., as senior engineer (supply). In 1948 he became assistant chief engineer (design) and then was promoted to deputy chief engineer (design) in 1950, and chief engineer (design) in 1953. He continued to hold this post on the formation of the U.K. Atomic Energy Authority a year later and in 1956 was appointed deputy director (reactors) and in 1957 deputy director (industrial power). In the following year he became director of industrial power, and in 1959, on the division of the Industrial Group, Mr. Cunningham became deputy managing director (technical) of the Production Group. He was appointed to his present post in April this year.

Mr. H. V. Disney, C.B.E.

Mr. Harold Vernon Disney, who is to succeed Mr. J. B. W. Cunningham as managing director of the Engineering Group of the U.K. Atomic Energy Authority, was born in Ruddington, Notts., on July 2, 1907. He was educated at Ilkeston and at University College, Nottingham. After apprenticeship with G. R. Turner, Langley Mill, Notts., Mr. Disney went as a designer to International Combustion, Derby, in 1931. He joined Imperial Chemical Industries (Alkali), Ltd., Northwich, in 1935 as a design engineer on chemical plant projects. In 1940 he was seconded by Imperial Chemical Industries for special duties on the construction of Ministry of Supply propellent and filling factories, and in 1944 became deputy director (filling factories supply). Mr. Disney was a member of the original team which started the Department of Atomic Energy at Risley in February 1946 and was appointed chief engineer, services and supply, in 1947. In 1950 he became chief design engineer for the diffusion plant at Capenhurst, and in 1957 was promoted to deputy director (defence plants) at the U.K. Atomic Energy Authority. He was appointed director of engineering of the Industrial Group in May 1958, becoming director of engineering of the Development and Engineering Group in July 1959 and deputy managing director of the Engineering Group in 1961. As from November 1, and until he takes over full responsibilities, he will be managing director (designate) of the Group.

Royal Society of Edinburgh : Officers for 1961–62

At the annual statutory meeting of the Royal Society of Edinburgh, held on October 24, the following officers and members of Council were elected: *President*, Prof. E. L. Hirst; *Vice-Presidents*, Dr. T. R. Bolam, Dr. Douglas Guthrie, Dr. A. W. Greenwood, Prof. R. A. Rankin, Dr. C. E. Lucas, Prof. J. A. Macdonald; *General Secretary*, Prof. Norman Feather; *Secretaries to Ordinary Meetings*, Dr. Mowbray Ritchie, Prof. A. E. Ritchie; *Treasurer*, Dr. J. R. Peddie; *Curator of Library and Museum*, Dr. R. Schlapp; *Members of Council*, Prof. H. A. Brück, Dr. H. R. Fletcher, Prof. G. L. Montgomery, Prof. W. L. Weipers, Prof. J. H. Burnett, Prof. C. F. Davidson, Prof. D. C. Pack, Dr. A. B. Stewart, Prof. Jack Allen, Dr. S. C. Curran, Dr. G. H. Mitchell, Dr. Magnus Pyke.

Jubilee of the Atomic Nucleus

There have been speculations on the nature of atoms since the days of the ancient Greeks, but the first firm foundation on which modern ideas are built was the nuclear theory put forward by Rutherford in Manchester in 1911. Jubilee celebrations and an international conference were held in Manchester recently, and now the Science Museum has set up a small group of exhibits to illustrate various theories of atomic structure and Rutherford's great contribution. The exhibits are on view in the Atomic Physics Section of the Museum. They include a group of models of atoms as visualized by J. J. Thomson before Rutherford's theory; a further group illustrating Rutherford–Bohr atoms; several historic photographs relating to Rutherford's great Manchester period of research; and a working mechanical model illustrating how the observed scattering of α-rays by heavy atoms led him to the inescapable conclusion that the atom must have a small 'hard core' (which is now known as the nucleus). This nucleus itself is now being investigated with all the powerful and elaborate apparatus of modern physics and engineering. The Science Museum exhibit makes no attempt to illustrate the many practical applications of our knowledge of the structure of the atom, as these will be dealt with in a full-scale special exhibition, "Atoms at Work," which will be staged in the Museum by the United Kingdom Atomic Energy Authority in March 1962.

Centenary of Plastics 1862–1962

A joint committee of the Plastics Institute and the British Plastics Federation has been set up to organize a number of events in connexion with a

Centenary of Plastics 1862–1962. It was at the International Exhibition in London in 1862 that Alexander Parkes showed for the first time to the public "a new material and manufacture which from its valuable properties had induced the inventor to patent the discovery in England and France and to devote his attention for the last 10 years to the development of the capabilities and application of this beautiful substance to the Arts". In the case shown at the Exhibition were a few illustrations of the numerous purposes for which, according to Parkes, 'Parkesine' might be applied. These included medallions, salvers, hollow ware, tubes, buttons, combs, knife handles, pierced and fret work, inlaid work, bookbinding, card cases, boxes, pens, penholders, etc. Parkes received a prize medal for his exhibit. 'Parkesine' contained no camphor, an indispensable component of the later material celluloid, and while Parkes mentioned camphor in his patent of 1865 he did not appreciate its unique properties. There can be no doubt, however, that 1862 saw for the first time the material which led directly to the modern form of celluloid. The programme to be organized by the Joint Committee will include open days in firms in the plastics industry and special meetings and lectures throughout the centenary year (1962–63), culminating in the International Plastics Exhibition to be held at Olympia during June 12–22, 1963.

Personnel Management Salaries

During the summer of 1956 the Institute of Personnel Management carried out a survey of salaries paid to members. This was undertaken because the Institute had been frequently asked by individual members, and by companies filling vacancies, about salaries paid to personnel officers and other members of personnel departments. To collect this information, questionnaires were sent to companies throughout Britain and senior personnel officers were asked to complete these on behalf of their personnel staff. A report based on this survey was issued at the beginning of 1957. This report proved to be of great value both to individual members and also to companies filling vacancies or setting up personnel departments. The Institute has now taken a further and more extensive survey to obtain an up-to-date review of members' salaries in Britain (Personnel Management Salaries: Report of a Survey undertaken in February/March 1961. Pp. 32. London: Institute of Personnel Management, 1961. 21*s*.). Of the 2,900 questionnaires sent out to practising personnel officers, approximately 1,250 questionnaires were returned, of which 1,222 were included in the final analysis. The personnel officers completing the questionnaire can be regarded as representative of the Institute members as a whole. The report consists of eighteen tables which give salaries paid under a number of different headings, including salaries according to positions held, age group, type of company, industry, area, length of time with the firm, and number of employees.

The National Mineral Collection, Ottawa

A National Mineral Collection, containing specimens from Canada and all over the world, has been established at Ottawa through the co-operation of two Federal Government departments. The Collection will consist of two series: a systematic reference series to be maintained by the Geological Survey of Canada, within the Department of Mines and Technical Surveys, with H. R. Steacy as curator, and a display series, in the National Museum, under the curatorship of Colonel E. Taylor. The Museum is within the Department of Northern Affairs and National Resources. A single catalogue, to be prepared and maintained by the Geological Survey, will cover the whole Collection. Both Departments already possess substantial collections of minerals accumulated since the Geological Survey was formed in 1842. The number of specimens in the keeping of the Survey is estimated at 50,000. Among them are rare gold nuggets and precious stones, as well as large pieces of silver—one of them 3½ ft. long—found by geologists, prospectors, and mining crews in many parts of Canada. The curators of both series will endeavour to expand and to improve their series year by year. The Collection now has specimens representing about 30 per cent of the world's known mineral types—a valuable aid to training and research in geological science and exploration.

Recent Developments in Medical Treatment

A welcome sign of how clinicians are adapting themselves to the changing outlook in medicine is shown in the current issue of the *Practitioner* (187, No. 1120; October 1961). This is a special issue dealing with advances in treatment, and three of the contributors do not concern themselves with 'treatment' in the narrow sense of the term. The contributors in question deal with advances in pædiatrics, general practice, and public health, the inclusion of the latter subject indicating that preventive medicine is an integral part of the practice of medicine. The reasons why the other two subjects have been dealt with in a particular way are given by the contributors themselves. "The Holistic conception of child life within the family circle", according to Prof. W. S. Craig, "is fundamental to pædiatric thought, and a knowledge of human relations, and of the art of consultation and physical examination, must continue to provide the foundations of pædiatric teaching". "Too often", according to Dr. Richard Scott, "advance is halted by our failure to apply existing knowledge. It would appear that, as in many other spheres, so also in medicine are we more skilful in advancing knowledge than in applying it". That 'treatment', in the narrower sense of the term, is not overlooked is well exemplified by the other fifteen articles in the symposium.

The Perkin Centenary Trust: Awards

Applications for the awards of the Perkin Centenary Fellowship and Scholarships are invited by the Trustees. *The Perkin Centenary Fellowship* is offered for one or two years for the purpose of higher study of any subject approved by the Trustees. Candidates will be required to show either that they have had experience in an industrial firm or other institution concerned in the manufacture or the application of colouring matters, or that their intended field of study has a direct bearing on these subjects. The value of the Fellowship is £750 per annum with an additional grant of up to £100 per annum towards certain designated expenses. It is tenable, from October 1962, at any university, technical college or other institution approved by the Trustees. *The Perkin Centenary Scholarship*: two such awards are offered, each for two years, to enable candidates employed in an industrial firm or other institution concerned with the manufacture or the application of

colouring matters to receive an education at a university or technical college. Each award will have a value of £350 per annum. This may be increased, at the discretion of the Trustees, to £400 per annum, if the candidate appointed is required to live away from his normal place of residence. Applications and inquiries should be addressed to the Secretary, Perkin Centenary Trust, c/o Chemical Society, Burlington House, London, W.1. Applications must be made by May 1, 1962.

The U.S. Building Research Institute

THE Building Research Institute, a constituent part of the Division of Engineering and Industrial Research of the U.S. National Academy of Sciences–National Research Council, will become an independent technical society for the building sciences next year. As Dr. Detlev W. Bronk, president of the Academy–Research Council, remarked: "The Buckley report, which recommended in 1951 the establishment of the Building Research Institute within the Academy–Research Council, recommended also that the Institute be encouraged to work toward independent status. It is truly gratifying that, within the span of a decade, the Institute has established itself as a uniquely effective instrument for the stimulation and correlation of interdisciplinary research in the building industry, the extraordinarily diverse interests of which otherwise depend on the attention of nearly 500 separate associations in the field".

Acoustical Society of America

DR. LEO L. BERANEK, president of Bolt Beranek and Newman Inc., of Cambridge, Mass., has been given the Wallace Clement Sabine Award of the Acoustical Society of America for his contributions to architectural acoustics. The Pioneers of Underwater Sound Award has been conferred on Dr. A. B. Wood, of the Admiralty Research Laboratory, Teddington.

The officers of the Society are as follows: *President*, Dr. Laurence Batchelder, Raytheon Co., Burlington, Mass.; *Vice-president*, Prof. Robert T. Beyer, Brown University; *President-elect*, Dr. Robert W. Leonard, University of California at Los Angeles; *Treasurer*, Herbert A. Erf, Cleveland, Ohio; *Secretary*, Wallace Waterfall, American Institute of Physics, New York; *Editor-in-Chief*, Dean R. Bruce Lindsay, Brown University.

Biological Engineering Society

AT the annual general meeting of the Biological Engineering Society, Prof. R. F. Woolmer was re-elected president for the coming year, and Dr. A. Nightingale, St. Thomas's Hospital, London, S.E.1, as honorary secretary. The following members were elected as members of council: P. E. K. Donaldson, D. Gordon, A. Nightingale, G. Pampiglione, W. J. Perkins, J. T. Scales, B. Shackel, A. M. Uttley, H. S. Wolff, B. M. Wright.

University News :

Birmingham

THE following appointments have been made: Dr. J. S. Brimacombe, to be lecturer in the Department of Chemistry; Dr. F. Rieband, to be lecturer in the Department of Mechanical Engineering; E. D. R. Shearman, to be senior lecturer in electromagnetism in the Department of Electrical Engineering; Dr. E. C. Grant, to be Research Fellow in the Department of Medical Biochemistry and Pharmacology.

Edinburgh

THE following appointments to lectureships have been announced: I. W. Sutherland (bacteriology); J. Shepherd (electrical engineering); W. Dudgeon (senior lecturer in engineering); J. A. Linnet (engineering); Dr. S. Soundranayagan (engineering); H. Montgomery (pathology); M. B. Smith (pathology); Dr. P. D. Mitchell (senior lecturer in zoology).

London

DR. J. CLUNIE, lecturer at the Imperial College of Science and Technology, has been appointed to a readership in mathematical analysis tenable at that College.

New York

NEW YORK UNIVERSITY has combined its two physics departments into a single all-University department and has appointed Prof. Bruno Zumino, professor of physics, as its head. The re-organization is one of the final steps toward unifying the departments at the several centres of this University. The two units involved in the change are the University Heights Physics Department, formerly in the College of Engineering, and the Washington Square College of Arts and Science Physics Department. Each of the physics units under Dr. Zumino's direction will continue to have its own chairman. Prof. Sidney Borowitz has been appointed chairman of the University Heights Department to replace Dr. Lyle Borst, who has assumed a professorship in the all-University Department of Chemistry. Prof. Morris H. Shamos will continue as chairman of the Washington Square College of Arts and Science Physics Department.

Announcements

THE Executive Council of the Commonwealth Agricultural Bureaux has appointed Dr. V. Armstrong, representing New Zealand, as chairman in succession to Mr. C. K. Reheem, representing Pakistan. Mr. W. G. Alexander, representing the United Kingdom, has succeeded Dr. V. Armstrong as vice-chairman.

DR. A. W. BALDWIN, formerly an associate research manager of the Dyestuffs Division, Imperial Chemical Industries, Ltd., has been co-opted as a governor of the J. A. Radley Research Institute, 220–222 Elgar Road, Reading.

DR. ROBERT PHYFE MADDEN, chief of the Spectroscopy Section, Physics Research Laboratory, in the Engineer Research and Development Laboratories at Ft. Belvoir, Va., has been appointed to the Atomic Physics Division of the U.S. National Bureau of Standards. His new group is to develop absolute radiation standards for the far ultra-violet spectral region.

THE ninth annual Western Spectroscopy Conference will be held at Asilomar, Pacific Grove, California, during January 25–26, 1962. Further information can be obtained from Dr. Donald G. Rea, chairman, Western Spectroscopy Association, Space Sciences Laboratory, Leuschner Observatory, University of California, Berkeley 4, California.

ERRATUM. In the communication by M. R. Droop entitled "Evidence for a Sexual Phase in the Life-Cycle of an Amœba" in *Nature* of October 21, p. 281, par. 2, lines 1 and 5, the names *Heteramœba* and *Heterochromonas* should be interchanged.

XUM

PHILOSOPHY OF SCIENCE

THE sixth annual conference of the British Society for the Philosophy of Science was held at St. Hugh's College, Oxford, during September 22–24. The four formal meetings and discussions, once again organized by Dr. Mary Hesse (Cambridge), were attended by eighty members and guests.

The theme of the first meeting, with Dr. B. A. Farrell (Oxford) in the chair, was explanation in psychology. The first of the two papers was read by Dr. M. Treisman (Oxford), who challenged the widespread belief that, whereas the physical and biological sciences use only public data, the psychologist also uses private data of immediate experience. Dr. Treisman's method was to select topics from the history of psychology to show that the modern psychologist has refined the vague descriptive analyses of the past and now uses concepts as precisely definable and as objective as those of the physicist. The first illustrative topic he discussed was the analysis of instinct. He contrasted McDougall's classification analysis, vague and merely descriptive, with the present-day model of instinctive behaviour in animals based on the work of Lorenz and Tinbergen. Here there was little to dispute though the argument edged close to the controversial issue of reductionism. His second topic was the history of the measurement of sensation: he described the experiments of Fechner and of Stevens and Davis which led to the apparently conflicting laws $S = K \log I$ and $S = K I^n$ respectively, where S is the 'measure of sensation' and I is the intensity of the stimulus. Dr. Treisman argued that these laws could be reconciled but that the second was preferable because it had more explanatory power.

Dr. J. O. Wisdom (London School of Economics) widened the scope of the kind of psychological explanation to be considered by discussing the hypotheses used by psychoanalysts to explain patients' associations. Such an explanation, he argued, requires to be tested clinically by observing the patient's response to a presentation of an interpretation embodying the hypothesis. But, following Seeborn Jones, he indicated that two problems then arise: Does the presented interpretation cause a change? Is the interpretation true? He proffered some new criteria to distinguish between an interpretation that causes a change by suggestion and an interpretation that is true.

A long and lively discussion ensued. The chairman began by challenging the assumption implicit in Dr. Wisdom's paper that the reports of the psychoanalyst could be regarded as objective data, but, for an hour, discussion was focused on Dr. Treisman's reconciliation of the two laws of sensation, a reconciliation which failed to satisfy the mathematicians present. Eventually there was a return to psychoanalysis: What are its basic statements? To what extent could the reports of the analyst about his subject be regarded as objective data? But again the discussion returned to the measurement of sensation. How could 'half a feeling' be measured?, the physicists demanded. Various solutions to the many problems raised echoed along the corridors of St. Hugh's until well after midnight.

At the next session the president, Prof. R. B. Braithwaite (Cambridge), took the chair for a discussion on learning machines. Dr. F. H. George (Bristol), treading delicately over this philosophically thorny field, outlined some of his recent work. He gave a lively description of his method of programming a *Deuce* computer so that one-half of the machine taught the other half to play an optimal game of noughts and crosses—a by no means trivial game, as the chairman had later occasion to emphasize. Dr. George went on to explain more generally how a computer can 'learn' to generalize from its own experience and to symbolize or 'name' the processes it goes through. He suggested that such performances give the computer qualities analogous to the insight and flexibility of human thought-processes.

Prof. D. M. Mackay (North Staffordshire) gave a thoughtful elaboration of Eddington's account of 'the two tables' extended to persons (and their brains) and to machines. He first considered the transition from the micro-description of a mechanism to its behavioural description in such terms as 'controlling' and 'goal-seeking'. He argued that such behavioural terms presuppose an under-specification of the physical situation which becomes meaningless when applied to mechanisms specified in atomic detail. He then extended his analysis to 'game-playing' mechanisms and explained how the crucial difference between playing against and manipulating a machine lies in the nature of one's commitment towards it. Finally, he extended his analysis to human beings, regarded as persons and as cell-mechanisms, and discussed the degree to which it is possible to switch from one view to the other when one is confronted by another human being.

In the discussion Dr. George was asked to elucidate some technical points, but members' questions mainly sought clarification of Prof. Mackay's concept of under-specification, its relation to entropy and its implications for personal commitment in dialogue.

The third meeting was devoted to a discussion of genetic coding and information theory, with Mr. F. T. C. Harris (Middlesex Hospital Medical School) as chairman. Dr. I. Leslie (Belfast) gave the first of two papers. Using a helpful series of slides, he reviewed with modest impartiality some recent developments in the biochemical analysis of the processes by which information is transferred from the genes to the cellular sites of protein synthesis. He explained the background to the present conflict of ideas about the way in which ribonucleic acid (RNA) acts as an intermediary in this transference. In the 'classical' view much of the RNA is in the form of stable templates which are coded by DNA for the synthesis of specific proteins: but recent experiments on bacteria and bacterial viruses suggest that some of the RNA acts as a 'messenger' which itself carries genetic information from the genes to the protein-synthesizing mechanisms.

The second main speaker, Dr. S. Brenner (Cambridge), remarking that "philosophers are not really concerned with the facts", bounded gaily from Dr. Leslie's well-laid factual foundations into genetic space. He delighted the audience with bold speculative leaps and a frank analysis of his own approach to the problem. He based his study of genetic coding, he explained, on two main principles: (1) that "any-

thing useless must be wrong", and (2) that "there is no security problem in Nature", that is, Nature is no enemy striving to conceal the truth from us. He then attacked the view that the stable RNA of the ribosomes acts as a template and argued that the ribosomes are structures which synthesize, at a given time, the protein dictated by the attendant messenger. Thus he rejected two- and three-process models of transfer of information in favour of a one-process model which he claimed was universal.

The discussion was at first confined to thrust and counterthrust between the two experts, deftly provoked by the chairman. But gradually the audience gained sufficient confidence to intervene. Questions about the evolution and the controlling mechanisms of this complex system (for example: How do you make a cat ?) led to lively discussions. It slowly became apparent that though genetic coding was concerned with transfer of information, information theory itself had little to offer in solving the problems of genetic coding apart from providing some descriptive terms. As Dr. Brenner put it in his final remark, "It's now just a matter of linguistics", that is, of reading the code.

The fourth and final discussion was devoted to philosophical aspects of cosmology. Prof. J. L. Mackie (Sydney) took the chair, and Mr. R. Harré (Oxford) and Dr. W. Davidson (Battersea College of Technology) were the two main speakers. Mr. Harré argued that the universe, defined as 'everything there is', was logically not a proper subject for scientific study. He carefully distinguished between the cosmo-sciences and the astro-sciences by analysing the logical form of typical propositions of these subjects; though cosmography, he thought, could be regarded as scientific, the other cosmo-sciences, cosmogeny and cosmophysics, were merely speculative. His main attack centred on the concept of infinity as used in or implied by the cosmo-sciences: he distinguished between the concept of an actual or referential infinity, which he rejected as logically improper, and the concept of a descriptive infinity which (analogously with the intuitionist concept of infinity in mathematics) has a finite starting point and a rule of progression which may continue without limit and thus be a growing but potentially infinite sequence.

Dr. Davidson argued that development of the cosmo-sciences was scientifically both valid and feasible, and that such questions as whether the universe is spatially infinite or not, or whether it had an origin in time or not, are not necessarily transcendental issues. He claimed that the unity of science as a whole may depend on the development of a satisfactory cosmology in which every science could find its place.

With the two protagonists thus apparently diametrically opposed, the chairman formulated their main differences and members of the Conference set about their task of resolving them. In the discussion, Mr. Harré's dependence on Kantian antinomies, Russell's theory of types, and *a priori* arguments took some hard knocks. It seemed eventually that perhaps only the philosopher's concept of the universe was referentially infinite, while the cosmologist's universe, if not finite, was at worst only descriptively infinite and therefore not, logically, a wholly improper subject of study.

The conference ended with a business meeting at which, *inter alia*, members were able to express their thanks to St. Hugh's College as hosts and to Dr. Mary Hesse as organizer for the parts they played in a pleasant and stimulating week-end.

B. C. Brookes

IONIC MELTS

LIQUIDS still constitute one of the least well understood states of matter. Near the critical temperature various quasi-gaseous formulations of their behaviour are often quite successful, but near the melting point quasi-crystalline models offer more promise. Ionic melts have been much less studied than many other liquids. Their critical temperatures are usually not known, but are certain to be high. For this reason, current structural models for ionic melts show a bias towards quasi-crystalline concepts, which is strengthened by the strong statistical probability that each cation will have anions as its nearest neighbours, and vice versa, in order to lessen electrostatic repulsion energy.

However, really precise theories of the structure of ionic melts present difficulties which have not yet been surmounted. In addition to the theoretical difficulties, until recent years the amount and diversity of experimental work on ionic melts has been restricted by technical complications. Temperatures of study need to range from 100° to well above 1,000° C., and many of the melts attack practically every kind of containing wall. Despite these obstacles, interest in the science and technology of ionic melts has been growing rapidly. Sessions dealing with these systems have been included in a number of recent international scientific meetings, and two Gordon conferences in the United States have been wholly devoted to discussing their properties. At the discussion meeting of the Faraday Society, held in the University of Liverpool during September 5–7, seventy-five scientists from Britain and fifty from overseas had the opportunity of comparing progress in three main fields: structure, thermodynamic properties, and transport properties of ionic melts. The president, Sir Cyril Hinshelwood, was in the chair. Prof. H. Bloom, of the University of Tasmania, gave the eleventh Spiers Memorial Lecture on "Structural Models for Molten Salts and their Mixtures".

A broad conclusion from the discussion was that interesting and important experimental work is being carried out in quite a few centres. Comprehensive theoretical treatments of the properties of ionic melts have by no means caught up with the range or the physical interest of the data now being obtained. Applied research on ionic melts in electrometallurgy or as heat-transfer fluids appears to be reasonably active in various countries. The number of substances capable of existence as stable liquids in the range 300–1,000° C. is comparatively small, which points to the profitability of exploring further fields of application of ionic melts. At present, basic research on ionic melts seems scanty in British universities,

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having regard to the importance of extending physico-chemical research to higher temperatures, and to the properties of condensed states of matter generally.

The first group of six papers presented at the Liverpool meeting of the Faraday Society were concerned with structure and spectra. In dealing with problems of structure, methods of describing and measuring the nearest neighbour and next-nearest neighbour environment of any ions continue to be of prime importance. Although they are indispensable, X-ray techniques applied to ionic melts suffer from the weakness that as with other liquids they only yield information in the form of radial distribution functions, which give average environments. In a significant number of ionic melts, there is evidence for a closer approach between anion and cation, and an increase of separation between ions of like sign, compared with the crystals. This shrinkage of contact distance between ions of unlike sign has been explained in terms of the increased positional disorder in the melt, compared with the crystal. Disorder results in less complete compensation of electrostatic forces from the nearest neighbour shell of unlike ions, exerted on the ion at the centre of the shell. Greater local polarizations can be expected in the melt, even when the total volume it occupies is larger than when the same quantity of matter is in the crystalline state. Data were presented for radial distribution functions of molten carbonates and sulphates.

There was considerable discussion about the local arrangements of ions in close-packed melts. Spectroscopic techniques promise to give rather more specific information about local structure than is obtainable from X-ray determinations of radial distribution functions. Experimental studies presented for discussion included ultra-violet absorption spectra of transition metal ions in dilute solution melts. These yield information about ligand fields around the transition ion. A somewhat different type of ultra-violet absorption spectrum arises from so-called charge transfer effects, which involve excitation of an electron in the anion to an orbital the radius of which depends on that of the shell of cations immediately surrounding the anion. Such spectra can therefore yield valuable information about the configurational changes in this shell of nearest neighbours. One paper dealt with the modifications of charge transfer spectra that accompany various changes such as thermal expansion, the transition from one crystal form to another, and the transition from crystal to melt. Thermal expansions of crystalline nitrates observed by conventional means are found to match quite closely the expansions of the charge transfer orbitals as determined by the ultra-violet absorption spectra. This makes it all the more striking that volume changes on melting, as determined by conventional methods, are in the opposite direction to the changes in the charge transfer orbitals. Compared with the crystals, optical evidence for the melts thus points to a greater proportion of more tightly packed local assemblies of ions. Since the thermodynamic evidence shows an increase in volume, presumably this expansion results from increasing the proportion of vacancies and holes on melting alkali nitrates. Rubidium nitrate is one of the very few crystals which shrink on melting. Even here, the shrinkage of the charge transfer orbitals appears to be greater than the average contraction observed thermodynamically. For nitrates and other salts of polyatomic anions that have low melting points it is claimed that spectral evidence supports the general theory that the melting mechanism of these crystals includes the formation of 'association complexes', as well as the more usual increase of positional disorder and randomization of orientation of non-spherical ions. A second paper on charge transfer spectra described changes in mixed molten nitrates in terms of the effective electrostatic fields due to neighbours and next-nearest neighbours. Interesting information about the structure of liquids seems likely to emerge from extensions of such studies on mixtures of molten salts.

There was considerable but not very conclusive discussion about the usefulness or otherwise of attempting experimental and even conceptual distinction between 'association complexes' and 'complex ions'. It seems to be generally agreed that complex ions can be readily distinguished when they are formed in dilute solution in ionizing solvents; many techniques for their identification have been developed since Arrhenius. In condensed ionic assemblies, whether crystalline or molten, the distorting influence of nearest neighbours can make identification of a complex much less discriminating. 'Association complexes' can be the result of various ways of packing ions so as to minimize their potential energy. In discussing melting mechanisms, it is important to recognize that some of these packings are 'non-crystallizable'; for these, addition of more and more ions according to the same rules of packing will not fill space economically. Only crystal nuclei can be extended by packing additional ions indefinitely in three directions in space; but so long as they are quite small, non-crystallizable association complexes may compete thermodynamically with potential crystal nuclei in the melt, which can be regarded as containing a mixture of the various kinds.

For discussing melting mechanisms, it may not be necessary to decide whether the reduction of potential energy on passing from random ionic packing to an association complex results primarily from electrostatic effects, or whether recognizable 'complex ions' are formed in which delocalization of valency electrons may extend over all the atoms in the complex.

But for the interpretation of other properties of ionic melts, the identification of strongly bonded complex ions may be more urgent. For example, cryoscopic measurements continue to yield suggestive structural information. One group of studies presented for discussion referred to the formation of complex ions such as $CdCl_2$ or $PbCl_3^-$ analogous to those encountered in aqueous ionic solutions.

A novel field is opened up by cryoscopic and other studies of the behaviour of solutions of metals in ionic melts. In some of these solutions the electron appears to leave the metal atom and may have a role analogous to that in solutions of alkali metals in liquid ammonia. Theoretical and experimental aspects of the electronic properties of such solutions received considerable discussion. Transport of electrons under the influence of electric fields applied to such systems has interesting contrasts and analogies with the movement of charge carriers in semiconductors. Conductances can be quite high even when the concentration of the metal atom is fairly dilute. Developments of these investigations promise to throw much additional light on conductance mechanisms generally that do not involve ion migration in condensed phases.

Papers presented at the meeting point to significant advances in the precision of measurement of basic

thermodynamic parameters of ionic melts, such as activity coefficients. Evaluations were reported of excess functions for the enthalpy and changes in volume on mixing ionic melts of the same or of different valency structure. As a result, some of the current quantitative theories that have been developed for non-ionic liquid mixtures can begin to be adapted to ionic melts. Considerable advances may be expected in the next few years in this branch of the theory of liquids.

One-third of the papers presented dealt with transport properties of ionic melts, which have been studied for a number of years and which continue to receive much attention. Use of radioisotopes has greatly enriched the possibilities of making measurements on diffusion, the correlation of which with other transport phenomena helps to unravel complicated structural problems. In addition to their technological importance, transport properties can yield tests of theoretical models for ionic melts that can be much more sensitive than equilibrium properties to structural details assumed in the model. Discussion of measurements of thermo-electric power of ionic melts suggested further interesting possibilities that seem likely to attract additional attention in the years ahead. A. R. UBBELOHDE

WOOD EXTRACTIVES

A SYMPOSIUM on wood extractives was arranged by the Plant Phenolics Group and held at the Royal Holloway College, Englefield Green, Surrey, during September 20–22. The meeting proved valuable in bringing together workers in different fields, including several from overseas, having a common interest in the minor or extraneous components of woods. After an introductory session the subject of wood extractives was dealt with under three headings, namely biosynthesis and taxonomy, the chemistry of extractives, and their influence on utilization of wood and wood products.

Dr. F. E. King (British Petroleum Co., Ltd.) opened the symposium with an introductory address entitled "Perspectives in the Chemistry of Wood Extractives". Dr. King surveyed the historical development of investigations on extractives, commencing with those of technical interest such as the tannins, dyestuffs and resins, and proceeding to the work of Erdtman on the heartwood components of coniferous trees and his own work on the corresponding components of hardwoods. These investigations led to the conception of heartwood components as 'taxonomic tracers' which can be used to supplement the observations on morphological features employed by botanists in the taxonomic classification of trees. Dr. King emphasized the wide variety of C_6–C_3–C_6 compounds that are found in woods and discussed a few unusual structures that have been encountered.

In the second paper, given by Prof. F. W. Jane (Royal Holloway College), an account was given of the structure of the tree and the distribution of the various tissues in it. This was helpful to the audience, consisting predominantly of chemists. The extraneous substances are classified by botanists as gums, resins, tannins, oils, mucilages, colouring matters, mineral deposits, etc., and the location of these in the structure was described and illustrated. There is clearly room for closer co-ordination between the observations of botanists, based largely on the microscopic staining reactions of these components, and their investigation by chemists.

Prof. E. J. Bourne (Royal Holloway College) described some new methods for the characterization and separation of extractives that have been studied in his laboratory. These include the use of molybdates and tungstates which form complexes with polyhydroxy compounds and can assist in their separation by electrophoresis, the addition of phenylboronic acid to the solvents used in paper chromatographic separations of these compounds, the use of boron trichloride under very mild conditions to identify families of extractives by removing substituent groups, and the fractionation of mixtures by means of a new liquid/liquid countercurrent extraction technique, which was demonstrated.

The session on biosynthesis and taxonomy opened with a paper by Prof. W. Sandermann (Bundesforschungsanstalt für Forst- und Holzwirtschaft, Reinbek, West Germany) on the biogenesis of terpenes in plants. Prof. Sandermann discussed the part played by mevalonic acid and its conversion into geranyl and farnesyl pyrophosphates, from which the more complex terpenes may be derived. He then described his work on the incorporation of labelled precursors in plants and showed how a distinction between alternative mechanisms can be made by these methods ; for example, the biosynthesis of α-pinene and thujone was shown to take place by an electrophilic substitution rather than by an addition mechanism. A series of terpenoid compounds has been found in teak and correlated structurally with the anthraquinones present in this wood.

The systematic distribution of phenolic substances in plants was discussed by Dr. E. C. Bate-Smith and Dr. T. Swain (Low Temperature Research Station, Cambridge) in a paper presented by Dr. Swain. The contribution that a study of the chemical components of plants can make to taxonomic classification was emphasized. One of the results of an extensive survey of the occurrence of the common phenolics is the recognition that plants in some families or higher groupings are devoid of the capability of synthesizing certain types of compound. In this connexion the occurrence of compounds containing vicinal trihydroxy groups and of ellagic acid in members of the Rosaceae was discussed.

In continuation of this theme Prof. C. W. L. Bevan (University College, Ibadan, Nigeria) presented a paper entitled "Wood Extractives as Tracer Substances in Taxonomy", and referred particularly to the results of a survey carried out in his Department of components of woods of the family Meliaceae. He also outlined the procedures followed in determining the structures of gedunin, isolated from *Entandrophragma angolense* and of khivorin from *Khaya ivorensis* and *K. grandifoliola*.

In the session on the chemistry of wood extractives four papers were presented, dealing respectively with the lignans, tropolones, stilbenes, and flavonoids, these being the principal types of phenolic compounds

that are found in wood. In discussing the lignans Prof. R. D. Haworth (University of Sheffield) pointed out that the basic structure common to all lignans comprises two phenylpropane units linked in the β-positions. The structure and stereochemistry of some of the naturally occurring lignans, of which about forty are known, was considered. Where ring closure has occurred, with formation of phenyltetralin or furano-structures, the creation of a new asymmetric carbon atom renders the stereochemistry complex. There is evidence that the lignan structure can be formed by oxidation of αβ-unsaturated phenylpropane compounds, such as ferulic acid.

A survey of the discovery and determination of structure of the tropolones found in various woods was given by Prof. J. Gripenberg (Institute of Technology, Helsinki, Finland). The distribution of the tropolones, which appear to be confined to the family Cupressaceae, was discussed and their general properties and synthesis described. It is interesting to note that all the nine known tropolones occurring in wood contain an isopropyl (or isopropenyl) group and appear to be closely related to the terpenes. A scheme by which they may be derived from carone has been proposed by Erdtman.

Dr. D. E. Hathway (British Leather Manufacturers' Research Association) discussed the occurrence of stilbenes in wood and referred to the recorded incidence of hydroxystilbenes in conifers and dicotyledonous trees. In a study of the extractives of the heartwood of *Eucalyptus wandoo*, 3:5:4′-trihydroxystilbene and its 3-glucoside were found to be present, and these compounds have been used as tracer substances in an investigation of the heartwoods of 60 species belonging to the *Longiores* sub-section of the genus *Eucalyptus*. This method confirms the existence of certain anomalies in the classification that have been suspected on other grounds.

The fourth paper in this group, dealing with the flavonoids, was prepared by Dr. R. A. Laidlaw and Dr. J. W. W. Morgan (Forest Products Research Laboratory). After a short account of the occurrence, function and nature of the flavonoids of wood, Dr. Laidlaw went on to consider three classes of these compounds, namely the leucoanthocyanidins, dihydroflavonols and benzylcoumaranones, in greater detail. Methods for determination of the structure and stereochemistry of the leucoanthocyanidins and their characterization as flavan-3:4-diols were discussed. Among the dihydroflavonols, dihydromorin, phellamuretin and keyakinol were selected for special mention. The naturally occurring aurones and hydroxybenzylcoumaranones were described and the behaviour of maesopsin (tetrahydroxybenzylcoumaran-3-one) on heating with alkali was discussed in detail.

The final section of the symposium consisted of two papers in which different aspects of the utilization of wood were considered. Dr. R. H. Farmer (Forest Products Research Laboratory) discussed the influence of extractives present in different timbers on some of their technically important properties and applications. A brief account was given of the manner in which the colours of woods (and the changes in colour occurring under certain conditions) and their natural durability are related to the chemical nature of the extractives present in them. Examples were then given of interaction between wood components and other materials with which the wood comes into contact. These include the inhibition of the hardening of unsaturated polyester resins by certain timbers, shown to be due generally to quinones or hydroxystilbenes, and the retardation of setting of cement by water-soluble sugars and tannins.

In the final paper Prof. B. Lindberg (Swedish Forest Products Research Laboratory) described the influence of extractives on the pulping of timber. Extractives can influence the sulphite or the alkaline pulping processes in various ways. Thus they may render the penetration of the cooking liquor into the wood more difficult, react with the cooking liquors and modify them, or condense with the lignin, preventing its dissolution. Resinous and fatty components may give rise to 'pitch troubles', and can cause difficulty in bleaching because chlorination renders them more lipophilic. R. H. Farmer

THE INTERNATIONAL SCIENTIFIC FILM ASSOCIATION

FIFTEENTH CONGRESS

THE fifteenth annual congress of the International Scientific Film Association was held at Rabat, Morocco, during September 14–20, 1961. This was the first time that the Association had met outside Europe. The congress was under the patronage of H.R.H. Prince Moulay Abdullah.

Of the Association's twenty-five member countries, sixteen sent delegates and there were also observers from Guinea, India, Indonesia, the Lebanon and Mali. The largest delegation of twenty-two people was from Great Britain, followed by the U.S.S.R. with about twenty.

All delegates, observers and friends enjoyed the gracious Moroccan hospitality. From Rabat, where all were well housed in the new Cité Universitaire, excursions were made to Casablanca, Fes, Meknes and Marrakech. As the sun shone without hesitation throughout each day on every day, expeditions were also organized to the delightful beach at Temarah.

In his address of welcome, M. Moulay Ahmed Alaoui, Minister of Information, Fine Arts and Tourism, expressed his country's pleasure at the honour being paid to Morocco as the scene of the congress. This indicated also the importance of the African continent in the building up of the modern world. For countries like Morocco, the cinema was not a diversion, but an important instrument in education and instruction.

This congress has always two aspects: one organizational, the other concerned with the problems of scientific film makers. The former provides the machinery whereby the film makers can get together and see and discuss films in three main sections: research, education and popular science. For some time there has been a feeling that the organization has needed tightening, and this congress took certain steps to do so. The new president is Mr. Edgar Anstey (Great Britain), who replaces Mr. Alexandre

Zgouridi (U.S.S.R.). The new honorary secretary is M. Mohammed Afifi (Morocco), who replaces Mr. John Maddison (Great Britain). It is Mr. Maddison's guiding hand in the main which has been responsible for the steady development in the influence and prestige of the Association. He has now been elected an honorary life member.

The thirty films shown to the Research Section were described by its president, Dr. R. Robineaux (France), as on a very high level. Five films from France, two from Western Germany, and one each from Italy, the Netherlands and Czechoslovakia were specially selected by the Section for commendation. At the discussions the special theme was new techniques. The French described some new tools to aid research. These included:

(1) The light helper (this is a free translation of *l'asservisseur de lumière*), an instrument which allows the time of placing of the camera on the desired part of the curve of spectral sensitivity of the film to be fixed exactly and automatically, without acting on the diaphragm and without having to worry about rectifying the time of placing during the forward movement of panning of the camera. It can be used in particular for taking shots automatically and slowly during daylight; for example, during the complete phase of a solar eclipse when there are enormous differences in light. The instrument is a kind of 'cybernetic' tool.

(2) The *brillancemètre*, which is incorporated in the camera to allow of instantaneous control of the mean brilliance of the image.

(3) Motor regulated by transistor, a motor with continuous current fed by 8-, 12- or 24-V. batteries, with transistorized regulation between 8 and 40 images a second, to which a small frequency emitter can be added to record sound on a separate magnetic tape at 24 or 25 images a second, even with differences in tension of several volts in the batteries.

There was also a description of an apparatus to take under-water pictures with automatic illumination with synchronized flash. It was developed primarily with biologists in mind, but has been of interest also to industrialists for the study of corrosion of submerged mechanical parts, and for the study of currents and their effects on public works and harbour installations.

Dr. G. Wolf, director of the Institut für den Wissenshaftlichen Film, Göttingen, described how the film was being used for research in agriculture. Evenly distributed rye was fed over an endless belt into the bar drum of a threshing machine. In it the stresses to which the grain was subjected were observed and gauged. With a circumference speed of the drum of 30 m./sec., the grain moves about two-thirds more slowly. The flow of the threshed grain follows with irregular density due to congestion in the entrance. An analysis was made of exactly what happens in the different parts of the threshing cage and productivity was increased. Similar film analysis was done on the cutting capacity and cutting quality of the lawn-mower and hedge-cutting machine, which have developed empirically.

Mr. L. Hallett and Mr. John Maddison, both of Great Britain, read a paper on the "Use of Time Lapse in Research".

Thirty-eight films were exhibited for the Education Section. Unfortunately, as the president, Prof. J. Jacoby (Poland), stated, there were no films from Great Britain or the U.S.S.R.: these had been sent but had been mislaid. Prof. Jacoby was highly critical of the films seen. He thought the quality was lower than that he had seen at any congress. However, special mention was made of the films from Eastern Germany, which were commended for the high overall quality of their entry.

Prof. Jacoby said that screening conditions had made it impossible to appreciate the full technical quality of 16-mm. films. He wanted national branches of the International Scientific Film Association to take special care about the selection of films in future.

Seventy-two films were shown to the Popular Science Section. These were also of uneven quality and, as the president, Mr. V. Tosi (Italy), said, there was a great need for a clearer statement of the role and nature of the popular science film.

Running at the same time as the congress, was a festival of popular science films which were shown nightly and were open to the public. These films were included in the viewing by a special jury set up to recommend the outstanding popular science films of the congress. The following films were selected: Great Britain, "Electron Microscopy", made by A.E.I.; Eastern Germany, "Robert Koch"; Bulgaria, "Life in a Drop of Water"; Czechoslovakia, "Secrets of Heredity"; Hungary, "Silver Threads"; France, "Thaumetopoea"; Rumania, "Coke"; U.S.S.R., "Colour TV"; Poland, "The Gulf of Polar Bears". The British film, "Wild Highlands", made by the British Transport Commission, was mentioned for the high quality of its production and two Japanese films, "Lubricating Oil" and "Ancylostoma" were commended for their high technical quality. The Moroccan Scientific Film Association, as host country, awarded a diploma to the Japanese for their entries as best fulfilling the aims of the Association.

There was a special session on television, at which the main speaker was Mr. Aubrey Singer (Great Britain), of the B.B.C. During the past year, a commission for the study of problems of the use of television has been working. Its secretary, Mr. Jean Le Harivel (Great Britain), presented a detailed report based on information received from eight countries.

Most countries have some kind of science programme which goes out generally in the evening and is intended for large audiences of adults. Several countries produce science programmes for schools. A few countries have used television for the education of adults. This is true of Eastern Germany, with its television academy (mathematics and chemistry); of Czechoslovakia; of France, which has started technical education programmes and which is planning a series for workers on the social significance of technological developments; and of the United Kingdom, which is planning for 1962–63 a series of topical education programmes to supplement existing technical courses. In the United States, "Continental Classroom" is a television programme for the teaching of teachers. It goes out at 6.30 a.m. to a weekly audience of 1,700,000 through 150 stations.

Mr. Jan Varossieau (Netherlands) of the Stichting Film en Wetenschap Universitaire Film Institute, described the use of closed-circuit television for the teaching of science in the universities. A van had been fitted out to tour the country. It carried a Philips compact 'Vidicon' transistor camera. This could be linked to monitors in the lecture hall.

The congress agreed that closer co-operation between film and television producers would be of mutual benefit, and that there was a great need for centralized information services.

Information was given of the detailed plan prepared by the Association for Unesco to facilitate the distribution of films to universities. The purpose is to prepare a select list of films which could be used in first- or second-year university courses and of which it is possible to supply copies. This is an experimental project and the number of subjects to be covered and the number of films to be selected is limited. In co-operation with the International Council of Scientific Unions, a list of subjects has been prepared, reported Mr. B. Chibnall (Great Britain) the director of the project. The list includes subjects in botany, zoology, geology, chemistry and physics.

The International Film Library has been set up in Brussels, during the past year. It is having some initial troubles, but the Belgian Government has given a generous grant and premises. Among the directors are Mr. Edgar Anstey and Mr. John Maddison (both of Great Britain).

Among other decisions taken at the congress were to investigate the possibility of compiling and having published an international multilingual standard reference journal and vocabulary of scientific cinematography, and to standardize film catalogues. An offer by the Moroccan Government to investigate the possibility of setting up a distribution centre in Africa for scientific films was accepted. Two newly elected member countries of the Association are the United States and North Korea. This brings the total of member countries to twenty-seven, with three corresponding member countries.

The new officers of the Association are as follows: *President*, Edgar Anstey (Great Britain); *Vice-presidents*, J. Jacoby (Poland), S. Okada (Japan), R. Whaley (U.S.A.), A. Zgouridi (U.S.S.R.); *Hon. Secretary*, Mohammed Afifi (Morocco); *Hon. Treasurer*, Vladimir Vaclavek (Czechoslovakia).

The editor of *Scientific Film*, the official organ of the Association, is Mr. Maurice Goldsmith, c/o The Scientific Film Association, 55*a* Welbeck Street, London, W.1. The 1962 congress is to be held in Warsaw, and the 1963 congress in Rome. M. Goldsmith

EDUCATION IN EMERGENT COUNTRIES

Formerly an outstanding educational administrator in New Zealand and now his country's Ambassador to France and permanent delegate to Unesco, Dr. C. E. Beeby presented an interesting paper on September 5 to Section L (Education) of the British Association for the Advancement of Science meeting at Norwich. The paper was based on Dr. Beeby's experience with the growth of educational services in newly developing countries, and especially Western Samoa, but also drew from his wide acquaintance with the evolution of educational systems in Western countries.

Dr. Beeby showed that, while it has taken England a hundred years to work out a system of universal primary education fairly well adapted to the needs of the community and to the varied capacities of the children in schools, a number of emergent countries is now trying to do the same thing in a decade. At first sight it might appear that, with Western aid and benefiting from Western experience, they could leap the century and establish straight away something approaching a good modern type of classroom without having to plod through the stage of dreary formalism that marked the first fifty years of the Western system of compulsory schooling.

The position is not as simple as this, and the 'expert' advising an emergent country on its primary school system finds himself in the position of a butterfly teaching the chrysalis how to fly. There is an intermediate stage which must be gone through before some of his advice is of much significance. Dr. Beeby became aware of this sixteen years ago when he had some responsibility for the control of two education systems that were, at that time, at least half a century apart. He found himself, without any sense of inconsistency, encouraging in Western Samoa the introduction of the formal educational practices which he had for a long time been combating in New Zealand. This led to the conception of stages of development in the life-history of an education system, stages through which all systems, at least of a certain type, must pass, and which, though they may be shortened, cannot be skipped.

The inability of a primitive type of school system to profit immediately from all that has been learned about modern teaching methods is in no way due to any innate inferiority of either children or teachers in emergent countries. It depends primarily on two professional factors—the level of general education of the teachers in the system, and the amount and kind of professional training they have received. On this basis the development of an education system could be roughly divided into three stages: (1) 'dame school' stage, where teachers are ill-educated and untrained; (2) stage of formalism, where they are ill-educated but trained; (3) stage of meaning, where they are relatively well educated and also trained. At the dame school stage the syllabus consists of little but mechanical drill on the three R's and the memorizing of relatively meaningless symbols. The unsophisticated and untrained teacher turns naturally to formal methods of teaching, and the real weakness of the teaching at the dame school stage is that it is confusedly and inefficiently formal. It has all the defects of formalism and none of its virtues; it is formalistic in spirit without having form.

At the other end of the scale the main characteristics of modern education, in spite of its failings, are the attempt to give the child a deeper and wider understanding of the meaning of the symbols with which he works. Practice in a good modern school is free, informal, and apparently 'natural'; but it is based on a complex and sophisticated structure of theory, and, to succeed, it demands teachers who are both well-educated and well-trained. This is a long step from the ill-educated and untrained teacher at the dame school stage, and in between must inevitably come the stage of formalism where teachers are still ill-educated but are trained. For sheer formlessness the only remedy is more form, however much one may dislike formalism as such in education.

The freedom and informality of the modern classroom depend on the teacher being sure of himself and of his subject-matter, and a teacher who is teaching at the very limits of his knowledge can have no sense of security if he or his class wander beyond the bounds of a tight official syllabus or the paste-

board covers of the one official text-book. The only questions he can allow are those he asks himself, for they are the only ones of which he can be sure of knowing the answers. So the classroom must become formal. At the stage of formalism, symbols have more meaning for children than at a dame school stage, but it tends to be a narrow and restricted meaning. Classroom facts and skills are thin disparate things that mesh poorly with one another and scarcely at all with the warm rich world of the child's personal life.

It is tempting to try to find a short cut from the dame school stage or the lower levels of the stage of formalism to the best of modern teaching methods, but there seems little hope of this if the difficulty to be overcome is really the meagre education of the teachers who are themselves the products of the schools one is setting out to reform. With help and advice, the stage of formalism can certainly be reduced in length, but the reform of a school system is a skilled and delicate business, where too much haste can create confusion, and where our own unanalysed experience may not always be the best of guides. The difficulty is increased by the fact that not all the teachers in a system at any moment have the same capacity to take up new and complex methods.

A new element of urgency has developed with the explosive demand for education in newly independent States that has been one of the striking characteristics of the past decade. The demand for education, indeed, has become a new political force in the world, a force that no democratic Government can long resist, and countries are sometimes driven to launch ambitious schemes of universal education before they have teachers with enough education to make them effective. Unless there is to be a great waste of effort and grievous disappointment with the results of popular education in emergent countries, there is urgent need for a scholarly study, from a new and practical angle, of the whole natural history of the educational system and of the factors that help or retard its progress from stage to stage.

EXPECTATION OF LIFE IN THE UNITED STATES

A RECENT report issued by the Metropolitan Life Insurance Co., New York, directs attention to the fact that the average length of life (expectation of life at birth) among industrial policy-holders reached a new record of 70·5 years in 1960*. This is slightly above the figure for 1959, and only half a year greater than that for 1955. But although recent increases have been limited, long-term gains have been very impressive. Five years have been added to the average length of life of these policy-holders since 1946, ten years since 1937, and twenty-four years since 1909. In 1879–94, the earliest period for which data are available, the average lifetime was only 34 years—less than half that for 1960.

The largest gains in expectation of life have occurred at the younger ages. Thus, at the age of 5, the figure for 1960 was 5·9 years greater than that two decades ago. At the age of 40, the increase since 1940 has been 4·3 years, while at 65 the average future lifetime of those insured has been extended 2·3 years to its present level of 14·2 years.

The longevity of American wage-earners and their families has increased more rapidly than that of the general population of the United States. In 1909, the expectation of life at birth of the industrial policy-holders was about six years less than that of the population as a whole. By mid-century the two groups were on a par; at present industrial policy-holders have a slight advantage.

The long-term achievements in the conservation of life are accentuated by the very low death-rates now experienced in early life. Mortality of the coloured policy-holders, particularly beyond childhood, is considerably higher than that for whites. Among females, the rates for the coloured are at least double those for the whites from age 23 to the end of the child-bearing period. Even though the difference is not as large for males, the ratio of mortality among them for the age-range 24–39 is more than 3 : 2.

Similarly, the average length of life is appreciably greater for white than for coloured policy-holders, and in each race is greater for females than for males. In 1960, the expectation of life at age 5 years was 70·7 for white females, compared with 66·2 years for coloured females; the corresponding figures for males were 63·8 and 61·9 years.

* Metropolitan Life Insurance Company. *Statistical Bulletin*, Vol. 42 (June 1961): *Voting Population at Record High. Recent Variations in Pneumonia–Influenza Mortality. Longevity of Industrial Policy-holders in 1960. Accident Death Rates Decreases in Most States.* Pp. 12. (New York: Metropolitan Life Insurance Company, 1961.)

THE AMERICAN ACADEMY OF ARTS AND SCIENCES

THE *Records of the American Academy of Arts and Sciences 1960–1961** includes, as usual, the list of officers and committees, lists of members (including those elected May 10, 1961) with alphabetical index, the statutes and charter of incorporation, and reports submitted at the annual meeting. Special interest is given by the valedictory address of the retiring president, Kirtley F. Mather, which, under the title "Assay and Perspective" reviews the activities of the Academy during the four years of his presidency before appraising the present position of the Academy and its responsibilities and opportunities during the next decade. While it was doubtful whether the fullest possible use had been made of the Academy's new house, Mr. Mather thought that the success in developing its publication *Daedalus* as a journal of ideas and opinion had been outstanding and the Academy had been most successful in its development of conferences on topics of wide importance and interest.

As regards research, 119 grants totalling 91,903 dollars had been made in the past four and a half

* American Academy of Arts and Sciences, Boston. Records of the Academy (including new members elected 10 May, 1961), 1960–61. Pp. 157. (Boston, Mass.; American Academy of Arts and Sciences, 1961.)

years, compared with 452 applications aggregating more than 500,000 dollars, and the Academy was seeking to increase the number and scope of its awards for recognizing excellence; as yet it had no such award in the social sciences corresponding with its Rumford Medals in the physical sciences, the Amory Prizes in the medical sciences and the Emerson–Thereau Medal in the broad field of literature. The Academy had made no public pronouncement during the period, but Mr. Mather was not satisfied that there had been a suitable theme, and he inclined to think that the Academy was doing what it should more effectively by providing the information to illumine the problems on which policy makers must make their decisions.

As regards the future, Mr. Mather said that the Carnegie Corporation had made a grant of 50,000 dollars for each of the next two years for the continuation of the Academy's conference and study programme. It would be necessary in the next four years to assess the value of the Academy's house and the function of the Academy in the State, but he thought that the Academy's outstanding opportunity and responsibility in the next decade lay in serving as a liaison between the community of practical affairs and the practitioners and scholars in the arts and sciences.

BRITISH COLUMBIA RESEARCH COUNCIL

AMONG the varied research items described in the seventeenth annual report of the British Columbia Research Council is one into marine borer control (Pp. 16. Vancouver: British Columbia Research Council, 1961). During 1960–61 the stations recording marine-borer breeding were increased to more than thirty as more companies engaged the Division of Applied Biology for protecting logs and wooden installations against losses by borers. By its larval inspection and log-scheduling service, the Division undertook the protection of more than 100 million feet of timber in sea-water storage for Canadian companies and a similar volume for a company in Puget Sound, where the intensity of marine-borer attack is the highest on the North Pacific Coast.

In instances where serious damage by borers would occur before the logs could be used, the rafts were treated chemically by a sprinkling system developed by the Council. During 1960–61 more than 100 sections of high-grade stock were treated in this way prior to hauling from Alaska to Puget Sound; a total of 10 million board feet of wood were chemically treated.

One of the outstanding achievements in this field in 1960 was the development of a sonic device for testing the internal condition of pilings. This unit accurately reveals even minor damage by borers in piling. The unit has been used for testing the soundness of piling in wharves on Vancouver Island and in Vancouver Harbour. Operating in conjunction with a diver, 130 piles a day can be examined.

Also developed during the past year was a technique for enshrouding individual piles so that they can be treated chemically to kill embedded borers. This method was developed to protect uncreosoted piles which are used in many logging operations on the coast and as fender piles on many wharfs. The shroud is all plastic to avoid corrosion in sea-water, and can readily be clipped on and off a pile without the use of hooks.

A number of field studies on *Bankia setacea*, the local teredo-like borer, is being made. These include the susceptibility of different species of wood to attack, the rate of loss by different species, the effect of water temperature on breeding, and studies on the borer itself.

The Canadian Uranium Research Foundation has recently made a grant of 10,000 dollars a year for investigation into the use of uranium compounds as toxicant additives to wood impregnants for marine borers.

RECRYSTALLIZATION OF LEAD AND ZINC SULPHIDES

By Dr. J. C. NIXON
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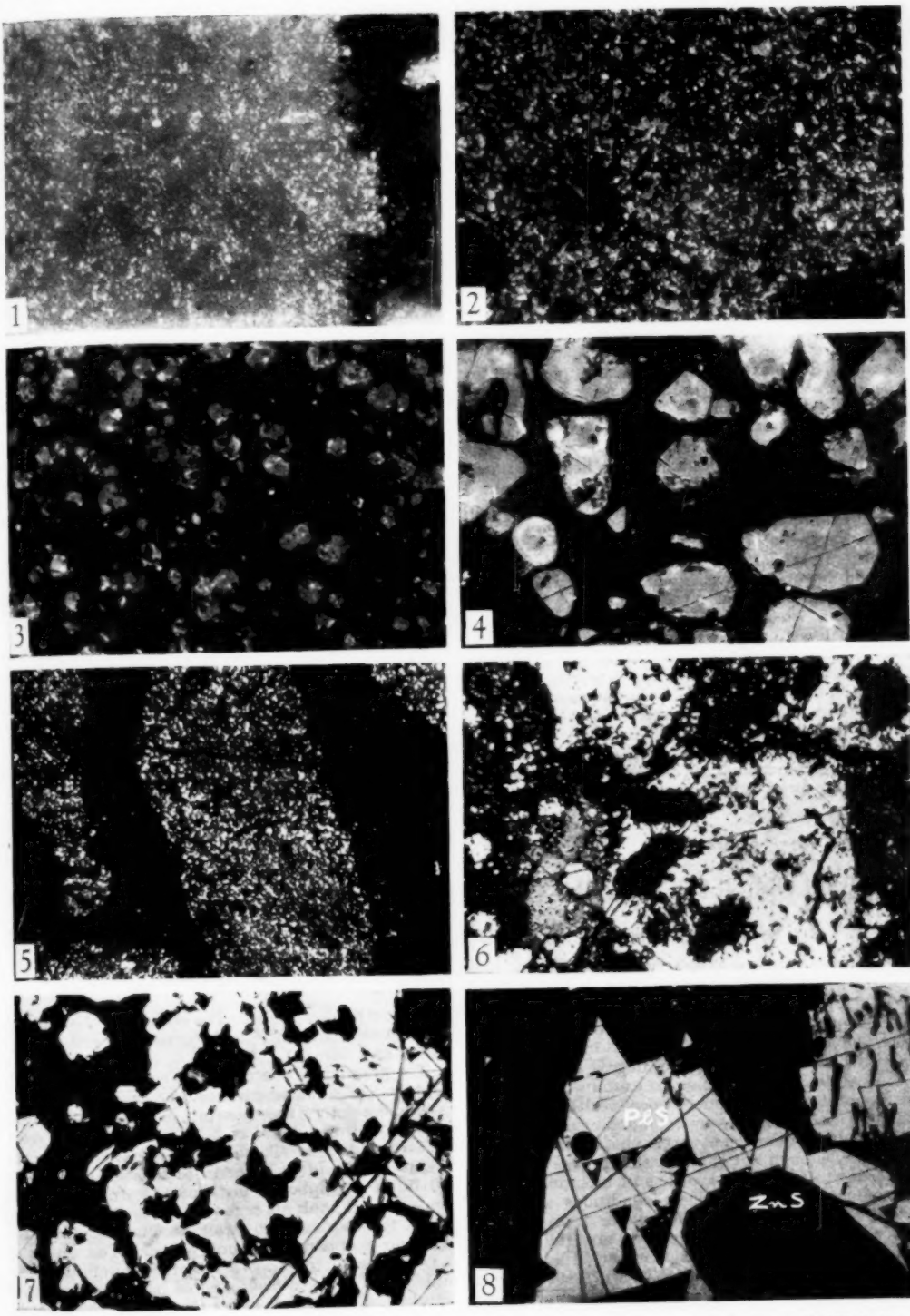
AND
J. D. HAYTON, D. C. LAWRIE and A. E. TYNAN
Australian Mineral Development Laboratories, Parkside, South Australia

IDEAS advanced in recent years for the formation of conformable ore deposits of base metals (the Broken Hill lead-zinc deposit in New South Wales is one of these) imply that the observed ore textures were developed by crystallization or re-crystallization after initial deposition. The earlier ideas depended in part on the assumption that the mineral textures were original and depositional. Since a useful understanding of the history of these deposits could be of assistance in exploration, some simple experiments were conducted to find out whether the textural condition of lead and zinc sulphides was or was not affected by temperatures which might be attained in deep burial and in high-rank metamorphism.

Chemically precipitated sulphides of lead and zinc were subjected to prolonged treatment in sealed vessels at elevated temperatures. The sulphides were prepared by precipitation with hydrogen sulphide from solutions of lead and zinc salts. The washed precipitates were dried at 90° C. in an inert atmosphere, and stored in a vacuum desiccator. Samples of each



Figs.: (1) Zinc sulphide heated at 200° C.; (2) heated at 400° C.; (3) heated at 600° C.; (4) heated at 800° C. (5) Lead sulphide heated at 200° C.; (6) heated at 400° C.; (7) heated at 600° C. (8) Zinc sulphide mixture (1 zinc : 1 lead) heated at 800° C. All at magnification 250

sulphide, alone, and with water were heated in a nitrogen atmosphere in stainless steel bombs for 3 weeks at temperatures of 200°, 400° and 600° C. A further sample of each sulphide with water and two mixtures of lead and zinc sulphides and water were heated (in bombs) at 800° C. for 3 days. The molecular ratios of zinc to lead were 1 and 2 in the respective mixtures. The temperature rose above 800° C. for part of the time—perhaps as high as 950°—and the bombs were so badly oxidized after three days that it was considered unsafe to continue heating. The initial sulphides and the materials discharged from the bombs were examined by X-ray diffraction and optical methods.

The X-ray patterns of the initial sulphides had broad peaks, indicating somewhat poor crystallinity. Patterns obtained for both sulphides after treatment at 200° C. for 3 weeks had sharper peaks, showing an improved degree of crystallinity. Materials from subsequent tests at higher temperatures gave diffraction patterns essentially the same as those obtained for the respective sulphides treated at 200° C., indicating that at this stage the materials were sufficiently well crystallized to show maximum sharpness in the diffractograph peaks. Products from heat treatment at temperatures higher than 200° C. were examined optically to determine the degree of crystallization. Optical examination revealed little significant difference between the initial sulphides and those treated at 200° C. In the latter it appeared that re-crystallization had begun in myriads of centres which were less than 1μ across in either sulphide. All these samples consisted of small granules, average size 2–3μ, which were aggregates of particles. Individual grains were detected in the materials heated at 400° C. and a few grains up to 80μ in width were seen in the zinc sulphide heated in the presence of water at this temperature. Some crystal faces were seen on the larger grains of this material. The zinc sulphides heated at 600° C. contained free grains showing no tendency to coalesce. The average particle size was approximately 12μ. The lead sulphides heated at 600° C. contained sintered aggregates of galena. The largest individual particles of galena in both samples were approximately 60μ across.

No really significant difference in resultant particle size was detected between wet and dry treatment. This would indicate that the higher pressures resulting from the presence of water vapour do not have any pronounced effect on the resultant particle size. The zinc sulphide heated at 800° C. for 3 days contained particles up to 90μ in width. The majority were between 30 and 50μ across, and well crystallized. The lead sulphide was reduced to metallic lead at 800° C. There are several possible reactions by which this could occur, but this aspect was not investigated. The lead sulphide in the mixtures was similarly reduced but there were some remnants of galena. In the mixture which contained the higher proportion of lead sulphide (1 zinc : 1 lead) many crystals of both sphalerite and galena were more than 0·5 mm. long and the galena contained numerous blebs of sphalerite typical of a eutectic textural intergrowth. The curved contacts between much of the two minerals were comparable with the mutual boundaries texture found in many natural galena–sphalerite ore bodies. Relatively more of the galena was reduced to metallic lead in the mixture which contained the lesser proportion of lead sulphide (2 zinc : 1 lead). However, the remnants of galena were well crystallized, but of smaller crystal size than in the other sample. The sphalerite was well crystallized and massive, with crystals of the same order of magnitude as in the other mixture.

It is clear that heating lead and zinc sulphides under the conditions described resulted in crystal growth, the effect being more enhanced at higher temperatures. The accompanying photomicrographs of polished sections of the briquetted materials illustrate this trend most effectively.

The work described was done by the Australian Mineral Development Laboratories on behalf of Consolidated Zinc Pty. Ltd.

BIOLUMINESCENCE IN THE SURFACE WATERS OF THE SEA

By RICHARD H. BACKUS, CHARLES S. YENTSCH and ASA WING

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

RECENT work by Clarke *et al.*[1–4], who have lowered photomultiplier tubes to great depths at sea, have shown that flashing by bioluminescent organisms is present in varying degree at all depths in all localities of the ocean so far examined. Clarke's instrument detects bioluminescence resulting from the combined activities of a variety of organisms and only measures bioluminescence when the ambient light-level is low enough not to mask the flashes.

In order to make observations of bioluminescence independent of ambient light conditions we have devised an apparatus in which water from the sea surface is pumped at the rate of six gallons per minute through a darkened chamber fitted with a photomultiplier tube (*RCA* 6655–*A* with *S*11 spectral response). The tube is connected by appropriate circuiting to a recorder such that organisms luminescing while passing through the chamber are tallied and their numbers recorded at the end of either a 5-min. or a 1-hr. period. The effluent of the chamber may be strained with plankton nets for collecting its fauna and flora.

The plumbing system (Fig. 1) consists of the pump (Jabsco 2187), plastic intake and discharge hoses ½ in. inside diameter, the viewing chamber (where the stream of water is about 2 in. wide and ¼ in. deep) and a flow-meter. The length of the intake hose was about 13 ft. in a harbour installation and about 50 ft. in a shipboard installation. In the harbour apparatus it took an organism somewhat more than 1 sec. to pass from the intake to chamber and in the shipborne apparatus about 5 sec. The intake of bioluminescent organisms into the plumbing system stimulates them to flash. Some flash at the point of intake and others all along the hose between intake and chamber, but, as our observations show, many flash in the chamber where the flow pattern is radically altered.

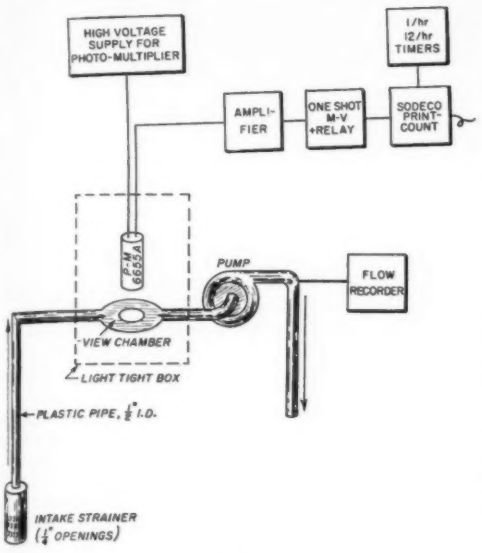


Fig. 1

At the shore installation counts were recorded by either a Hewlett–Packard print-out counter (Counter 523 *BN* and Printer 560–*A*) or a Sodeco 1*Tpb*53 print-out counter. At sea, counting was done with a simple solenoid-operated Sodeco counter which was read at appropriate times and re-zeroed.

Counts of flashes for 1-hr. periods for five days each in May and November 1960, in Great Harbor, Woods Hole, are shown in Fig. 2. A pronounced diurnal rhythm in activity is obvious. In May, maximum, or night-time, counts were about 700 times the minimum, or day-time counts, while in November these differed by a factor of about 100. Details of the rapid increase in count which occurs at sunset are shown in Fig. 3.

Table 1. Bioluminescence Counts (Period Totals) and Numbers of Organisms (per m.³ of Effluent) in Great Harbor, Woods Hole, December 2, 1960

Time (E.S.T.)	Bioluminescence	Diatoms*	Dinoflagellates†	Tintinnids*	Copepods‡
1330–1500	52	35,600	1,425	3,560	2,140
1730–1900	6,120	156,200	6,500	4,130	3,440

* Not luminescent.
† All (?) species luminescent.
‡ Some species luminescent.

Table 2. Station Descriptions

Station	Position	Date (1960)	Time (E.D.T.)	Depth* (fm.)	Surf. Temp. (°F.)
A	40° 39′ N., 72° 30′ W. to 40° 35′ N., 72° 41′ W.	Sept. 20	1230–2115	19–21	66·5
B	40° 29′ N., 72° 40′ W.	Sept. 21	0110–0130	24	62·8
C	40° 20′ N., 72° 39′ W.	Sept. 20–21	2310–0010	25	64·0
D	40° 06′ N., 72° 37′ W.	Sept. 21	0420–0445	29	—
E	39° 35′ N., 72° 32′ W. to 39° 23′ N., 72° 45′ W.	Sept. 21	1115–2100	44–50	65·4
F	38° 55′ N., 72° 34′ W.	Sept. 22	0055–0130	920–970	73·5
G	38° 28′ N., 72° 27′ W.	Sept. 22	0425–0450	1,470–1,475	74·8
H	37° 35′ N., 72° 15′ W. to 37° 26′ N., 72° 23′ W.	Sept. 22–23	1215–0730	1,680–1,760	71·9

* Bioluminescence observations and plankton samples were all taken from 1 m. under the surface.

Here counts every 5 min. are plotted relative to sunset for two days each in May and November 1960 and show that prior to sunset more bioluminescence occurred in May than November.

A No. 20 plankton net was used to strain the effluent for 1½ hr. at the time of minimum count and again for the same period at the time of maximum count on December 2, 1960. Counts of the principal groups found and the bioluminescence counts are given in Table 1. The numbers of organisms deemed potential sources of bioluminescence increased at

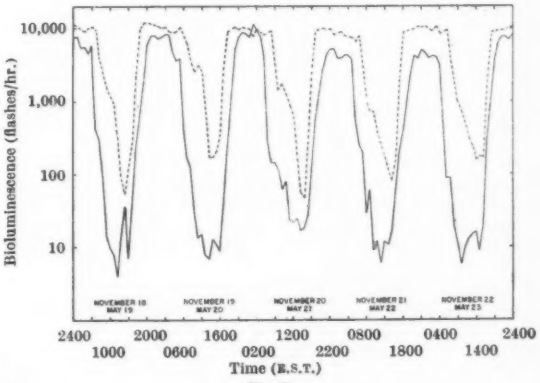


Fig. 2

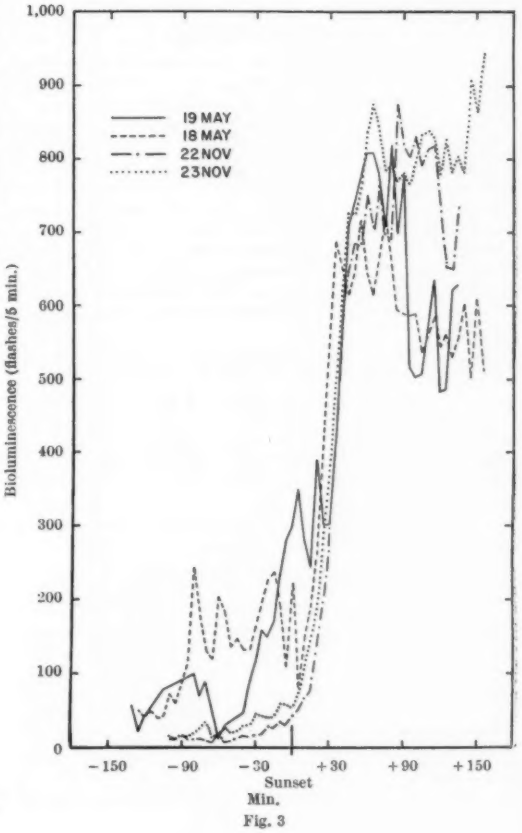


Fig. 3

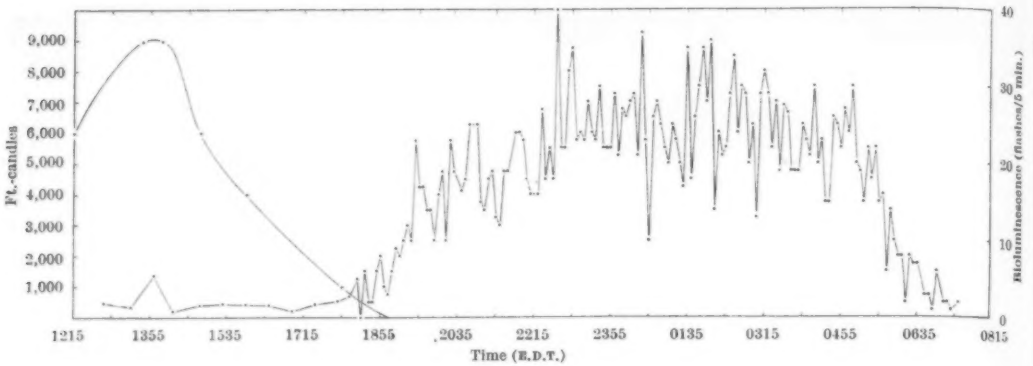


Fig. 4

night by factors of about 1·2–5. While a part of the increase in numbers of organisms in the night sample may be due to vertical migration, the species composition was quite different from that during day, which suggests that the water mass changed between sampling times.

Similar observations of bioluminescence were made at eight stations (Table 2) in the continental shelf and slope waters south of New England in September 1960. The plankton community was collected simultaneously by passing the effluent of the pump through a No. 20 plankton net. Three stations (*A*, *E* and *H*) were occupied for 6–10 hr., including the time of sunset, in order to study the diurnal variation of bioluminescence. The other five were used with these three for studying the geographical changes in night-time bioluminescence over the onshore–offshore section.

At stations *A*, *E* and *H* a diurnal rhythm in bioluminescence was observed similar to that described for Great Harbor. The observations for station *H* are shown in Fig. 4. Here, as at stations *A* and *E*, sharp increases in counting-rate were observed when the daylight intensity fell below about 500 foot-candles. This appeared to be true regardless of the magnitude of radiation during the day. Bioluminescence counts and counts of the principal organisms found in the effluent at station *H* during the afternoon and evening are shown in Table 3. Even considering the errors that may acrue due to plankton-counting techniques and to the loss of smaller organisms through the net it seems improbable that we would miss a vertical migration of plankton consistent with and of a magnitude sufficient to account for the diurnal changes in bioluminescence observed. Results at both stations *A* and *E* were similar.

Table 3. Bioluminescence Counts (hourly totals) and Numbers of Organisms (per m.³ of effluent) at Station *H*

Time (E.D.T.)	Bio-lumin-escence	Diatoms	Dino-flagel-lates	Green flagel-lates	Tin-tinnids	Copepods
1400–1500	15	1,600,000	3,063	700,000	396	6,030
1600–1700	16	1,150,000	1,975	300,000	1,088	1,877
1800–1900	46	567,000	2,322	437,000	838	4,440
1900–2000	143	1,475,000	3,510	271,000	811	3,780

Bioluminescence counts, counts of principal plankton organisms and amounts of net and total ('Millipore'-filtered) chlorophyll from the pump effluent during night hours at all stations are given in Table 4. Bioluminescence was at a maximum at *A*, the station nearest the coast, was low across the continental shelf (*B*–*D*) and rose to an intermediate value at the edge of the shelf (*E*) and remained moderately high at the stations in slope water (*F*–*H*). Numbers of dinoflagellates (mostly genus *Ceratium*) more or less followed this trend and it is probable that most of the bioluminescence we observed came from these organisms. The copepods were more numerous in slope water than at the stations on the shelf, but the cladocerans were present at the shelf stations and absent in the slope water. Tintinnids were absent at the shelf stations and present at the slope stations. The principal non-luminescent organisms encountered were diatoms. They were numerous on the shelf and, in general, diminished seaward except for the large population at station *H*. The distribution of total chlorophyll and net chlorophyll was also higher on the shelf than on the slope.

It has been our experience that the larger dinoflagellates, which are important producers of bioluminescence, are missed when counts are made on raw sea-water samples of 100–200 ml., thus a fine plankton net had to be used to concentrate these

Table 4. Bioluminescence, Principal Plankters (number per m.³) and Chlorophyll (μgm./l.) at Stations *A*–*H*

Station	Sampling period, E.D.T. (biolumin-escence)	Biolumin-escence* counts/5 min.	Sampling period, E.D.T. (plankton and chlorophyll)	Dino-flagellates	Copepods	Diatoms	Tintinnids	Cladocera	Chlorophyll† Total	Chlorophyll† Net
A	1955–2115	33·2	2000–2100	514,000	1,710	32,600,000	—	2,020	0·36	0·0021
B	0110–0130	12·5	0108–0128	842,000	1,161	54,000,000	—	327	0·56	0·0063
C	2310–0010	1·9	2352–0012	2,000	1,183	53,000,000	—	312	0·37	0·0060
D	0420–0445	4·8	0420–0444	1,470	1,770	1,019,000	—	—	0·32	0·0020
E	1955–2100	16·0	2000–2100	4,270	5,400	8,010,000	344	98	0·21	0·0006
F	0055–0130	16·9	0113–0133	4,160	5,055	6,540	1,800	—	0·39	0·0018
G	0425–0450	12·7	0425–0445	1,839	4,500	3,150	1,300	—	0·21	0·0016
H	1955–2200	18·1	1900–2000	3,510	4,000	1,480,000	811	—	0·20	0·0013

* Flow-rate, 25 l./min.
† Chlorophyll determinations by the methods of Richards with Thompson (ref. 5) and Creitz and Richards (ref. 6).

organisms. We are aware that a great fraction of the phytoplankton passed through the No. 20 net[11]. Comparison of the net to total chlorophyll (Table 4) shows that the net entrained less than 1 per cent of all the photosynthetic autotrophs.

Our observations are consistent with certain observations (summarized by Harvey[8]) of bioluminescence in sea-water samples containing dinoflagellates held under various conditions of light and dark, and with recent observations of circadian rhythms of bioluminescence in laboratory cultures of dinoflagellates[9,10].

Seliger, Fastie and McElroy[7] have recently published observations of bioluminescence in Chesapeake Bay using an instrument which integrates light intensity over short periods rather than counting flashes. They observed no diurnal rhythm and concluded that changes in intensity throughout the water column were due to the vertical migration of organisms. In contrast, we feel that the diurnal pattern of bioluminescence observed by us largely results from the inactivation of the bioluminescent system of the organisms responsible by daylight. The discrepancy between the Chesapeake Bay and Woods Hole results may be due in part to the difference in instruments but more than likely comes mainly from the geographical difference.

This work was supported by the National Science Foundation (grant *NSF*–G9579 and grant *NSF*–G8339) and the Atomic Energy Commission (contract *AT*(30–1)–1918).

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RESERPINE-INDUCED CIRCULATORY FAILURE

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During work on various drugs affecting adrenergic mechanisms, the first results of which have already been published[1], it was found that 24 hr. after the administration of 1 mgm./kgm. of reserpine a good number of cats, despite all care, died. Those animals which survived were very ill, lay immobile, unresponsive to sound, apparently lifeless. When handled and gently caressed, however, they opened their eyes and, with great difficulty although not paralysed, succeeded in making some movements, while purring and yet crying simultaneously. Despite all precautions, body temperature tended to fall, respiration was depressed, blood pressure was very low and the heart-rate markedly reduced. There was a loss of body-weight, possibly because the animal neither ate nor drank and, in addition, suffered marked diarrhœa. In other words, cats thus treated were very sick animals and presented a picture of great misery very similar in many ways to that of a human being during a stage of advanced shock (for example, late stages of acute circulatory failure), and definitely very much unlike the picture of an animal under the influence of an ordinary central depressant drug.

Reserpine is known to reduce or deplete the various tissues of their catecholamine content, and its pharmacological actions, especially those on circulation, have been, as a rule, attributed to this depletion[2]. Because of this, the cardiovascular system of animals pretreated with reserpine or isolated tissues from these animals have been extensively used for the study of the mechanism of action of various drugs. The cat has been a favourite experimental animal and, in order to 'reserpinize' it, doses of at least 1 mgm./kgm. of the drug have usually been used. The severe clinical condition, however, of the cats treated with reserpine was considered unlikely to be the result only of a depletion, however complete, of the animal's catecholamines. Experiments were therefore undertaken in which various parts of the cardiovascular system could be studied simultaneously *in vivo*.

Most cats were given a single dose of 1 mgm./kgm. of reserpine, either subcutaneously or intraperitoneally, on the day before the experiment. A few received the same amount of the drug spread over two or more days. Both the control and the reserpine-treated animals were anæsthetized with chloralose and prepared for the recording of blood pressure, peripheral blood flow (measured by recording the venous outflow from a hind limb) and force of cardiac contraction (recorded by means of a strain gauge arch attached directly to the muscle of either the right or the left ventricle).

The blood pressure of the cat pretreated with 1 mgm./kgm. of reserpine was low, rarely exceeding 70 and very often less than 50 mm. mercury and the heart-rate very slow. When the chest was opened, the heart was found to be enlarged, flabby and soft and the superior and inferior venæ cavæ and their tributaries engorged. The right side of the heart was, as a rule, at this stage more affected than the left. Moreover, the force of cardiac contraction was well below normal and the application of pharmacological tests clearly demonstrated that the heart was in failure. Furthermore, it was found that: (*a*) the sensitivity of the peripheral vessels to adrenaline, noradrenaline and isoprenaline, administered intravenously or close-arterially, was decreased; (*b*) any changes of blood pressure were, as a rule, secondary to changes in cardiac contraction; and (*c*) the peripheral blood flow passively followed the changes of blood pressure. In other words, 24 hr. after the administration of 1 mgm./kgm. of reserpine the cat exhibited a most spectacular circulatory failure, and furthermore any improvement of the circulation at

this stage was found to be almost exclusively the result of an amelioration of the force of cardiac contraction.

Although circulatory failure was always present, its degree varied from animal to animal. For example, in older, lactating or pregnant animals the condition was at its worst. When the heart failure was less advanced, the force of cardiac contraction increased after the administration of adrenaline, noradrenaline or isoprenaline. However, as a rule, noradrenaline produced the largest inotropic effect, adrenaline was less effective, followed by isoprenaline. Moreover, in a number of experiments the force of cardiac contraction after isoprenaline was either unaffected or clearly depressed. Thus, in the animals treated with reserpine the ratio appeared quite different from that in the control cats, where isoprenaline is the most powerful of the three catecholamines. The reason for this rather puzzling behaviour of the catecholamines in the animal treated with reserpine became apparent when changes of heart-rate were recorded at the same time. It was found that the heart of the animal treated with reserpine, in contrast to the control, was unable to sustain an increased activity. Consequently, its response to isoprenaline varied according to its condition at the moment of the administration of the drug; if very poor, the heart faced the abrupt increase in heart-rate with a clear-cut depression in the force of its contraction; on the other hand, when failure was not very advanced, the heart contraction could still improve, but even then the response was always much smaller than that usually seen in the control heart. A similar variation, although less pronounced, was found with adrenaline. In the majority of experiments, adrenaline did produce a positive inotropic effect. Occasionally, however, when heart failure was very advanced, the effect of adrenaline on the heart was depressant. The only catecholamine the administration of which was always followed by a positive inotropic effect was noradrenaline.

In control cats a dose of 50–100 μgm. of ouabain produced very little or no change at all in either the force of cardiac contraction or the blood pressure. In contrast, in the animals treated with reserpine after ouabain, a gradual increase in the amplitude of the cardiac contraction occurred accompanied by a simultaneous rise in blood pressure. Furthermore, ouabain was found partially to restore the positive inotropic effect of various drugs, which in the animals treated with reserpine became ineffective or depressant to the heart. This was the case with both tyramine and isoprenaline.

Furthermore, histological examination of the heart muscle revealed in each case extremely severe and widespread degenerative changes (Fig. 1). The examination of various other organs is still in progress. However, the results already obtained indicate that the liver undergoes, at this stage, centrilobular (fatty) changes indicative of heart failure and raised venous pressure. Moreover, while the adrenal medulla appeared normal, the cortex in most animals showed a picture characteristic of severe stress with widespread degenerative changes affecting the cells of both zonæ fastigiata and fasciculata.

From these results it is quite obvious that, in the cat, reserpine in a dose of 1 mgm./kgm. produces a most spectacular poisoning of the heart muscle, leading rapidly to disastrous functional impairment. As the myocardium is of the very essence in the maintenance of the circulation, all other factors

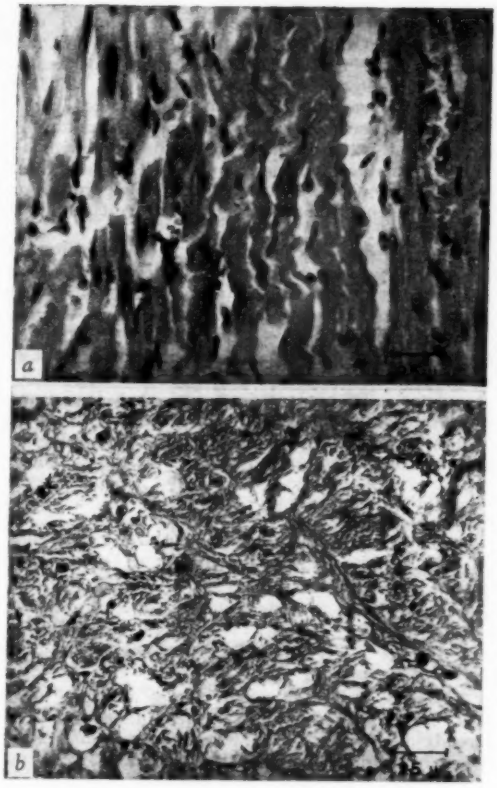

Fig. 1. Photomicrograph of myocardium (hæmatoxylin and eosin): (*a*) from a control cat; (*b*) from a cat treated with reserpine (0·25 mgm. per kgm. for 5 days)

which contribute to the circulation of the blood, although important, are likely to be secondary to the function and integrity of the heart muscle. However, the reduced sensitivity of the peripheral vessels of these animals to the normal mediators must undoubtedly contribute to the circulatory debacle which develops in such an incredibly short period of time.

The function and histological abnormalities found in the other organs of the animals treated with reserpine may be due to either direct toxic effects or to changes secondary to tissue anoxia, bound to exist in an acute circulatory failure.

The mechanism by which reserpine produces such powerful toxic effects remains to be elucidated. However, the functional and histological changes on one hand and the finding on the other hand that a metabolically 'active' tissue is more affected than a tissue relatively 'at rest' strongly suggest that the drug inhibits either some energy-yielding or some energy-consuming reaction. Kirpekar and Lewis[3] and Gillis[4] have already suggested a depressant effect of reserpine on the oxidative metabolism of various tissues.

Evidence that reserpine is a highly toxic drug has been previously reported. Unfortunately, such evidence has not received the attention it deserved. Saffran and Vogt[5] found that in rats a single intraperitoneal injection of reserpine (2·5 mgm./kgm.) produced a fall in the concentration of corticotrophin

of the pituitary to 30 per cent of the resting value. The authors reached the conclusion that this effect of reserpine is not due to interference with storage of adrenocorticotrophic hormone in the tissue, but was simply the result of the drug acting as a stressing agent. In their experiments, the losses in adrenocorticotrophic hormone were of a degree not previously encountered after the injection of drugs, but of the magnitude reported after adrenalectomy, or after removal of one and enucleation of the other adrenal. The only substance which in Saffran and Vogt's hands caused an even greater loss of adrenocorticotrophic hormone was a severely toxic drug—a nitrogen mustard; 24 hr. after the injection of di(2-chloroethyl) methylamine hydrochloride ('Mustine', Boots) the rats which had survived exhibited œdema and weakness and the corticotrophin of the pituitary had fallen to 10 per cent.

Goldin *et al.*[6] have reported that reserpine exerts an anti-tumour effect. They demonstrated that treatment with reserpine produced a significant increase in the survival-time of mice carrying advanced lymphoid leukæmia (*L* 1210) and caused regression of the local tumour which develops at the site of inoculation. Robison *et al.*[7] have made similar observations. Furthermore, West *et al.*[8] demonstrated that reserpine, in small doses, inhibited the growth of three different tumours in mice, and decreased uptake of oxygen in the intact animal. They reached the conclusion, however, that the anti-tumour effect of reserpine is mostly non-specific and dependent on depression and reduction of food intake.

Reserpine appears thus to be one of the most powerful 'poisons' known in pharmacology, and the 'reserpinized' animal, with both central and peripheral circulation in acute failure, a most abnormal experimental tool.

I am grateful to my colleagues, Drs. P. P. and M. G. Scott and Elizabeth Davis for their valuable advice and help with the histological studies. This investigation was supported by a grant from the Palissade Foundation, Inc., of New York, and grant *B*-2474 (*C*1) from the National Heart Institute, National Institutes of Health, U.S. Public Health Service.

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4-SULPHANILAMIDO-6-METHOXY AND 6-ETHOXY PYRIMIDINE (I.C.I. NOS. 32,525 AND 33,536): TWO NEW LONG-ACTING SULPHONAMIDES

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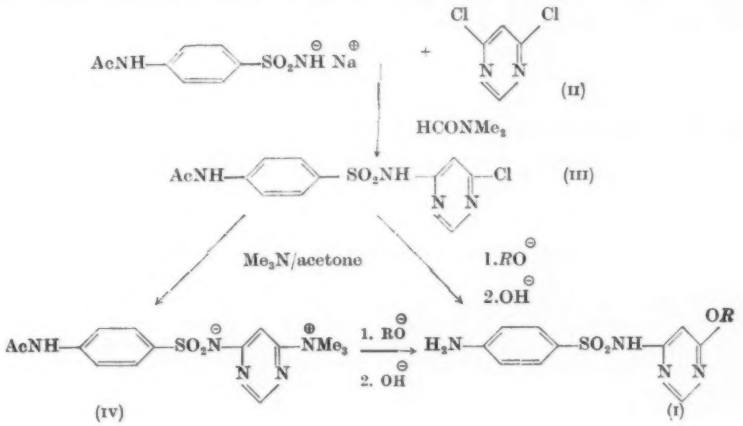
RENEWED activity in the sulphonamide field over the past few years has been aimed primarily at compounds with prolonged action. The first compound with this characteristic, 2-sulphanilamido-4,6-dimethoxypyrimidine, was synthesized in these laboratories in 1947 (ref. 1), and illustrated the importance of the methoxyl group in lowering the rate of excretion. Sulphamethoxypyridazine[2] and sulphadimethoxine[3] followed from the laboratories of American Cyanamid and Hoffmann–La Roche and have attained wide clinical use.

The one compound, closely related to these, which had not been prepared previously, was 4-sulphanilamido-6-methoxypyrimidine (I, R=Me). This and analogous compounds were readily available by the following route, starting with 4,6-dichloropyrimidine II (ref. 4):



The zwitter-ionic trimethylammonium compound (IV) proved to be a useful intermediate in the preparation of higher homologues (compare ref. 5) since it is much more reactive to nucleophilic reagents than is the chloro-compound (III). 4,6-Dichloropyrimidine (II) reacts smoothly with ethanolic ammonia at room temperature to give 4-amino-6-chloropyrimidine. Replacement of the 6-chlorine with alkoxide followed by the standard sulphonylation procedure yielded I. The experimental details of these reactions have already been published[6]. The synthesis of the methoxy compound by similar routes was afterwards published by the American Cyanamid workers[7].

The 6-methoxy (32,525) and 6-ethoxy (33,536) sulphonamides are of outstanding efficacy against experimental infections in mice, as shown here; but this effect falls off as the size of the alkoxy group increases, presumably because of poor absorption from the gastro-intestinal tract. Substitution in the 5-position of the pyrimidine ring also lowered the antibacterial effect in most cases[8].

The antibacterial action *in vivo* and *in vitro* of 32,525 and 33,536 is compared here against three most widely used, long-acting sulphonamides. The effect of sulphaphenazole[9] against an experimental *Streptococcus pyogenes* infection was so poor that this compound was not included in further comparisons. The three organisms used in this work, both *in vivo* and *in vitro*, were *Salmonella dublin*, *Streptococcus pyogenes* (strain Kruger) and *Staphylococcus aureus* (strain Buttle 147).

Bacteriostatic action in vitro. Median inhibitory concentration of the sulphonamides mentioned were determined in Jewell and Pearmain's medium[10] (median inhibitory concentrations are expressed in μgm./ml. and are the mean of three concordant observations, determined graphically); they are shown in Table 1.

Table 1

Sulphonamide	Test organism: *Strep. pyogenes*	*Staph. aureus*	*Sal. dublin*
32,525	13	3	5
33,536	19	3	15
Sulphadimethoxine	6	3·5	64
Sulphamethoxypyridazine	42	14	14
Sulphaphenazole	12	6	60

The results given in Table 1 show that 32,525 is as effective as the other compounds against the Gram-positive organisms and clearly illustrates its superiority against the Gram-negative.

Therapeutic action in vivo. Infected mice were treated orally with the sulphonamides, doses being given 1 hr. after the infection with subsequent doses at 24 and 48 hr. Deaths were recorded daily, mice alive on the eighth day (fifteenth in the *Staph. aureus* infection) being arbitrarily awarded a survival time of 8 (or 15) days. The figures given in Tables 2, 3 and 4 are 'effects', that is, mean survival time in days of the treated group minus mean survival time of the control group.

Table 2. *Streptococcus pyogenes*

Compound	Dose (mgm./20 gm.): 8	4	2	1	0·5
32,525	5·0	3·3	2·6	1·1	0·7
33,536	4·9	4·5	3·6	1·6	0·6
Sulphadimethoxine	3·7	1·2	0·4	0·2	0·0
Sulphamethoxypyridazine	3·8	2·3	1·8	0·6	0·2
Sulphaphenazole	1·9	0·4	0·2	0·0	0·0

Table 3. *Salmonella dublin*

Compound	Dose (mgm./20 gm.): 0·5	0·25	0·125	0·06	0·03	0·015
32,525	5·5	5·0	4·8	3·5	2·3	2·0
33,536	5·1	4·9	3·9	2·6	2·1	0·9
Sulphadimethoxine	2·9	2·3	0·7	0·0	0·9	—
Sulphamethylpyridazine	3·0	2·6	1·4	1·6	0·9	—

Table 4. *Staphylococcus aureus*

Compound	Dose (mgm./20 gm.): 0·5	0·25	0·125	0·06	0·03
32,525	12·5	11·1	8·5	4·7	1·0
33,536	10·9	10·9	2·7	1·4	—
Sulphadimethoxine	7·8	3·6	—	—	—
Sulphamethylpyridazine	10·9	2·1	1·3	0·0	—

It can be demonstrated that the fraction of a sulphonamide which is bound to plasma proteins has no antibacterial activity[11]. Equilibrium dialysis experiments between buffer and human (or mouse) plasma show that the two new compounds are less extensively bound than the older ones. This fact, together with the high intrinsic activity and a persistence in the mouse about equal to that of sulphamethoxypyridazine, probably explains the superior effect of 32,525 and 33,536.

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GROUP RETENTION FACTORS IN THE GAS-LIQUID CHROMATOGRAPHY OF STEROIDS

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IT has been shown[1] that the retention time of a mono-unsaturated sterol methyl ether on the gas-liquid chromatogram, in which polyethylene glycol succinate is the liquid phase, may be expressed in the form:

$$r_{(s+x)} = r_s \times k_x \qquad (1)$$

where $r_{(s+x)}$ is the retention time of the sterol having a double bond in position x, r_s is the retention time of the parent saturated structure and k_x is a constant factor, characteristic of the position of the double bond but independent of the molecular weight. The further relationship:

$$r_{(s+x+y)} = r_s \times k_x \times k_y \qquad (2)$$

describes the retention time, $r_{(s+x+y)}$ of a dienol methyl ether with non-interacting double bonds in positions x and y, the second double bond being characterized by the factor k_y.

These observations suggest that this type of relationship is a general one such that the introduction of a substituent group into a specific position in the steroid molecule influences the retention time by a factor which is a constant for the type of grouping and its particular site of attachment, but which is independent of the molecular weight of the total structure and of the influence of other functional groups, provided no interactions occur with them.

If this is so, it is to be expected that equation (2) may be applied in its most general form to the steroids, so that the retention time of a poly-substituted steroid in which intramolecular group interactions are negligible can be expressed as:

$$r_{(n+a+b+c+\cdots)} = r_n \times k_a \times k_b \times k_c \times \cdots \qquad (3)$$

where $r_{(n+a+b+c+\cdots)}$ is the retention time of the total structure, r_n is the retention time of the unsubstituted nucleus and $k_{a,b,c,\cdots}$ are group retention factors for a series of non-interacting substituents at positions a, b, c, etc. It is the purpose of this communication to present the preliminary results of some work with compounds which have been selected with the specific object of testing equation (3).

The steroids were authentic samples which had been synthesized by standard methods. Methyl ethers were prepared by reaction of the appropriate hydroxy-compounds with methyl iodide in the presence of potassium *t*.-butoxide in diethyl ether. Gas-liquid chromatography was carried out with 5 per cent polyethylene glycol succinate as the liquid phase under conditions exactly as previously described[1].

The retention times, relative to that of cholestane, of a series of steroid hydrocarbons, their 3β-methoxy-, 3β-methoxy-Δ^5-, and 5α-hydroxy-derivatives are presented in Table 1. In the last three columns of Table 1 are listed the retention factors for the 3β-methoxyl and 5α-hydroxyl groups and the Δ^5-bond, respectively. These are the separation factors between the saturated parent hydrocarbons and their corresponding 3β-methoxy- and 5α-hydroxy-derivatives and between the saturated 3β-methoxy-compounds and their Δ^5-derivatives.

The retention factor for the 3β-methoxyl group in the saturated series is seen to be the same, within the limits of measurement, in all cases. The factor associated with the introduction of the Δ^5 bond into the 3β-methoxy-compounds, which has already been shown to be constant for the C_{27}, C_{29} and C_{29} sterols[1], is seen from the present work to have the same value in the C_{19} and C_{21} series. The retention factor of the 5α-hydroxyl group shows greater variation than can be accounted for by operational error but still remains constant over a range of 4 per cent as between the pregnane and stigmastane derivatives.

Table 1. Relative Retention Times of some Steroid Hydrocarbons and Derivatives

Hydrocarbon	Substituents: 3β-methoxyl	5α-hydroxyl	3β-methoxy-Δ^5	Retention factors: 3β-methoxyl	5α-hydroxyl	Δ^5
Androstane 0·12	0·42	—	0·461	3·55	—	1·09
Allopregnane 0·21	0·73	1·10	0·806	3·50	5·25	1·10
Cholestane 1·00	3·50	5·24	3·80	3·50	5·24	1·09
Ergostane 1·38	4·89	6·98	5·22	3·54	5·06	1·07
Stigmastane 1·72	6·11	8·70	6·60	3·55	5·06	1·08

Table 2 presents some results obtained with a series of bifunctional steroids, chosen to test the accuracy with which retention times may be predicted by means of equation (3). In the left-hand side of the table are listed a series of monofunctional compounds and their retention times relative to that of cholestane. In the right-hand side a series of bifunctional derivatives are listed, together with their observed relative retention times and their calculated relative retention times derived from the data for the monofunctional steroids in the left-hand side of Table 2 and the appropriate values shown in Table 1. It is seen that in all except the last two cases (5α-hydroxy-Δ^2-cholestene and 5α-hydroxy-Δ^7-ergostene) there is close agreement (within 4 per cent) between the observed and calculated values.

Table 2. Observed and Calculated Relative Retention Times (r) of some Mono- and Bi-functional Steroids

Monofunctional steroids	r (obs.)	Bifunctional steroids	r (obs.)	r (calc.)
17β-Methoxyandrostane	0·333	3β,17β-Dimethoxy-androstane	1·19	1·18
7α-Methoxycholestane	1·67	3β,7α-Dimethoxy-cholestane	5·65	5·83
7β-Methoxycholestane	2·25	3β,7β-Dimethoxy-cholestane	7·65	7·88
		3β,7α-Dimethoxy-allopregnane	1·19	1·22
		3β,7β-Dimethoxy-allopregnane	1·60	1·64
7α-Hydroxycholestane	6·83	3β-Methoxy-7α-hydroxy-allopregnane	5·20	5·00
Δ^2-Cholestene	1·20	5α-Hydroxy-Δ^2-cholestene	5·34	6·30
Δ^7-Ergostene	1·80	5α-Hydroxy-Δ^7-ergostene	7·26	9·06

The aberrrant results obtained in the case of unsaturated 5α-hydroxy-compounds bear on the question of intramolecular group interactions. The calculated values for the relative retention times of 5α-hydroxy-Δ^2-cholestene and 5α-hydroxy-Δ^7-ergostene exceed the observed values by 18 and 25 per cent respectively. Measurements of Dreiding models indicate that the approximate distances of 3·1 and 2·9 Å. between the 5α-oxygen atom and C_2 and C_3 respectively in the Δ^2-compounds are almost identical with the distances between the oxygen atom and C_8 and C_7 in the Δ^7-compound. It is therefore reasonable that approximately the same degree of interaction should take place between the double bond and the homoallylic hydroxyl group in each case. The precise nature of the interaction is not clear. It may be in part a simple steric effect in which the hydroxyl group partially blocks the interaction between the double bond and the liquid phase but probably also involves a transannular π-bonded interaction between the double bond and the labile hydroxylic hydrogen. An evaluation of the relative importance of these two possible effects would require a study of analogues in which the 5α-hydroxyl group was replaced by an inert substituent of similar size.

These results attest to the validity of equation (3) as a general expression of the relationship between structure and retention time in the steroid series. They are entirely consistent with the relationships between members of the same and closely related homologous series which have already been firmly established by the work of James and others[2]. It

has been in the nature of the materials which have been most thoroughly investigated hitherto that emphasis has fallen more heavily on the logarithmic relationship between retention time and molecular weight within a homologous series than on these separation factors between different homologous series. In the present context the retention factors arising from the introduction of individual substituent groups into the molecule inevitably claim complete attention.

As was pointed out previously[1], the precise influence of the liquid phase on the values of the group retention factors remains to be established. Lipsky and Landowne, in a valuable summary[3] of separations achieved with a wide range of steroids on a variety of the less polar polyester liquid phases, have reported separation factors which are approximately of the same relative order as those obtained in these Laboratories. Similar results have also been reported by Haahti *et al.*[4]. It seems likely that the same general relationship expressed in equation (3) must hold with the less polar liquid phases but that the actual values of the group retention factors differ considerably according to the chemical nature of the liquid phase. In view of the generally shorter retention times obtaining with the less polar polyester liquid phases used by the above authors it is evident that these are to be preferred for the separation of highly oxygenated compounds since the cumulative effects of highly polar substituents soon lead to excessive retention times. For the analytical separation of simple sterols, in the form of their methyl ethers, however, there may well be some advantage in the use of the highly polar polyglycol succinate used in the present work.

The extension and further refinement of this type of study seem likely to yield information which will supplement in a novel manner the existing body of knowledge concerning reactivity of substituent groups and their intramolecular interactions. The practical value of the group retention factor method for the elucidation of hitherto unknown steroid structures is already established by its routine use in these Laboratories.

A complete account of this and related work will be presented elsewhere. I wish to express my thanks of Prof. Louis Fieser and Prof. H. B. Henbest for generous gifts of steroid samples and to Prof. Konrad Bloch for his interest.

This investigation was carried out during tenure of an established investigatorship of the American Heart Association and was supported by grants from the American Heart Association and the National Institutes of Health.

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SURVIVAL OF *RHIZOBIUM* ON CLOVER SEEDS

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THE high death-rate of *Rhizobium* inoculated on the surface of clover seeds is a serious problem where the survival of adequate numbers of rhizobia on the seed is essential for satisfactory nodulation. as in acid soils where the acidity further reduces the number of viable rhizobia[1]. On lime-coated seed, which is used for establishing legumes on acid soils[2], the death-rate is also high[3] and in addition the seed is difficult to re-inoculate. This article reports the survival of *Rhizobium* on seeds of subterranean clover for periods exceeding twelve months, in the presence or absence of a lime coat.

A treatment designed to force rhizobia under the seed coat[4] was tested in experiments with the Mount Barker strain of subterranean clover (*Trifolium subterraneum* L.). The method used was to immerse the seeds in a liquid culture of *Rhizobium*, and apply a vacuum of 700 mm. of mercury for three successive periods. The total period of immersion was 5 min.

Rhizobium trifolii strain *NA*30 was cultured in a yeast manitol medium[5] of *p*H 7. Immediately prior to inoculation, maltose or sucrose was added to the culture (nodulation was not affected by the form of sugar) to give a concentration of 10 per cent sugar. Solutions containing 10 per cent sugar were used for all dilutions and for the uninoculated controls. One set of replicates was prepared without a lime coat, and a corresponding set with a lime coat, using 50 mgm. of methyl ethyl cellulose[6] plus 5 gm. of precipitated calcium carbonate per 25 gm. of clover seed. The seeds were air-dried and stored in paper envelopes at room temperature, which seldom varied outside the range of 20°–27° C.

Two lots of seed which had been prepared separately (Exps. 1 and 2, Table 1) were sown on an agar medium of *p*H 7 in test-tubes 64 weeks after treatment. There were twelve tubes per treatment, with two seeds in each tube. Table 1 shows clearly that the use of the immersion and vacuum treatment enabled rhizobia to survive on a proportion of the seeds for 64 weeks, and that lime-coating the seeds had no harmful effect on this survival.

The results presented in Fig. 1 show the beneficial effects of the immersion and vacuum treatment in

Table 1. Nodulation of Subterranean Clover sown on an Agar Medium 64 Weeks after Treatment

Inoculation treatment		Percentage of tubes with nodulated plants	
Method	No. of rhizobia per ml. of inoculum*	Without lime coat	With lime coat
	Experiment 1		
Uninoculated	0	0	0
Normal	$2{\cdot}2 \times 10^7$	0	0
Immersion and vacuum	$2{\cdot}2 \times 10^8$	8	17
Immersion and vacuum	$2{\cdot}2 \times 10^9$	17	17
	Experiment 2		
Uninoculated	0	0	0
Normal	$2{\cdot}4 \times 10^8$	0	0
Immersion and vacuum	$2{\cdot}4 \times 10^9$	25	17
Immersion and vacuum	$2{\cdot}4 \times 10^9$	42	42

* For the normal inoculation treatment, 1 ml. of inoculum was added to 25 gm. of seed (approximately 3,000 seeds). For the immersion and vacuum treatment 25 gm. of seed absorbed about 3 ml. of inoculum.

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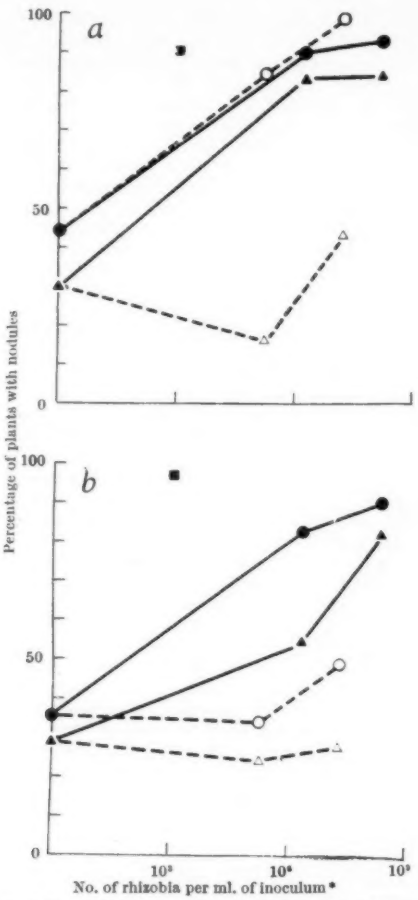

Fig. 1. Effects of treatments on the nodulation of subterranean clover sown on an acid soil in the field. *a*, 1 day; *b*, 12 weeks after inoculation. △, normal inoculation; ○, normal inoculation, with lime coat; ▲, immersion and vacuum inoculation; ●, immersion and vacuum inoculation, with lime coat; ■, freshly inoculated, drilled with lime. * See corresponding footnote to Table 1

field sowings on a virgin soil of *p*H 5·4. The normal method of inoculation was ineffective on this soil when used without a lime coating, even when the seeds were sown one day after inoculation. When the seeds were lime-coated the normal method of inoculation was effective for the first sowing, but was not effective after storage for twelve weeks. When the seeds were immersed in *Rhizobium* inoculum and subjected to vacuum, the inoculation was effective at both sowings. At the twelve-week sowing the highest percentage nodulation occurred where freshly inoculated seed was drilled with lime, but pre-inoculation by immersion and vacuum treatment combined with lime coating was nearly as good.

At the first field sowing (Fig. 1) there was a good response to inoculation by the immersion and vacuum treatment in the absence of a lime coat, suggesting that this method of inoculation had provided the *Rhizobium* with some protection against the harmful effects of the acid soil. The response was also evident at the twelve-week sowing, although here a lime coat was needed for the best response to inoculation where the lower number of rhizobia was used.

Further tests were made in an acid soil in a glass-house. All the treatments shown in Fig. 1 were included in a trial in pots sown 32 weeks after the treatment of the seed. In this trial the only response to inoculation was obtained where the immersion and vacuum treatment was used in conjunction with a lime coat on the seed. The lime-coated, vacuum-inoculated seed gave 75 per cent nodulated plants at each level of inoculation, compared with less than 20 per cent of nodulated plants for all other treatments.

At a 52-week sowing in pots, where the immersion and vacuum treatment was tested only in the presence of a lime coat, the lower and higher levels of inoculation respectively gave 16 per cent and 51 per cent nodulated plants, compared with no nodules on the uninoculated controls.

These results show that it is possible to inoculate clover seeds in a way that enables the rhizobia to survive for very long periods. It is clearly important to define the factors concerned in the effect of the immersion and vacuum treatment used, and in the survival of the rhizobia.

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CHOLINERGIC NEURONS IN VERTEBRATES

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CAVANAGH and Holland[1] have recently reported histochemical evidence showing that acetylcholinesterase (AChE) is more widely distributed in the nervous system of the chicken than in the nervous system of mammals. They found that in the hen, in contrast to mammals, it was present in the cytoplasm of all central neurons examined and was in the dorsal root ganglion cells as well. Because cholinergic cells characteristically contain AChE (ref. 2), they suggest that all neurons in this species are cholinergic and that in this respect it differs fundamentally from mammals, in which non-cholinergic as well as cholinergic cells are found. The additional possibility is adumbrated that an all-cholinergic nervous system is simpler than one to which non-cholinergic cells contribute and as such may be better suited to the requirements of a species less highly developed than mammals. I would like to comment on these suggestions in the light of earlier published work[2] and of evidence which I have been

collecting, as material becomes available, on acetylcholine (ACh) metabolism in representative vertebrate species. This later work, which is referred to here, has been briefly summarized in an article now in the press[3].

First, it is relevant to consider how far biochemical data can be used as criteria in identifying cholinergic neurons. Known cholinergic cells of the periphery are found to contain both AChE and ChAc (choline acetylase, the enzyme that synthesizes ACh). By combined histochemical and biochemical analysis it can be shown that the AChE is present both in the perikaryal cytoplasm and in the axoplasm of cholinergic neurons and, in addition, is concentrated at their axon terminals, although at this site a proportion of enzyme is postsynaptic and apparently the product of the cholinoceptive cell which they innervate. Where this characteristic disposition of AChE is observed it is highly probable that the cells in question are cholinergic. However, the presence of enzyme in the cell body does not necessarily mean this since the cytoplasm of certain non-cholinergic neurons, including some sympathetic and spinal ganglion cells, also contain AChE in fairly high concentration. Therefore, in order to find out if a cell is cholinergic, it is of greater importance to establish whether ChAc which has not been found in any proved non-cholinergic neurons is also present in it. Unfortunately, there is no suitable micro- or histo-chemical technique by which to determine the cellular location of ChAc, so that in samples of the central nervous system it is usually not possible to identify the neurons from which it is derived and the information obtained from its measurement is quantitative only. In the central nervous system, expressed as the amount of ACh which can be synthesized per gm. tissue per hr., its concentration may vary in some species of mammal from 0 to 5 μgm. in tissues such as the optic nerve and parts of the cerebellar cortex to amounts equivalent to an activity of 3 mgm. in the caudate nucleus; while in birds an even wider range of activity is found.

These variations of ChAc have been interpreted to mean that the proportion of cholinergic to other neurons varies in different parts of the central nervous system; and that its concentration is larger or smaller depending on the numbers of such neurons. To some extent it may also depend on which parts of them, the cell body, axon or axon terminals, are sampled since it appears that there is more enzyme at the axon terminals than elsewhere in the cell.

In line with this interpretation all central structures containing ChAc have AChE associated with it; and in the case of some tissues containing a high concentration of ChAc it has been possible to show that the distribution of AChE in some of the constituent neurons conforms to what would be expected if they were cholinergic. In much of the brain and spinal cord it is not possible, however, to apply this test because of the difficulty of tracing neurons through to endings that are often remote from the cells of origin. A new approach which helps to overcome this difficulty by determining the effect of chronic section on AChE in some central pathways has recently been described[4]; but at present, conclusions about the identity and course of cholinergic neurons in the central nervous system are largely a matter of inference.

The ultimate test of whether a nerve is cholinergic is the demonstration that it releases ACh to transmit impulses to the cell or cells with which it synapses. There is now evidence to show that ACh is released in the central nervous system, and in the case of the cerebral cortex it has been demonstrated that the rate at which this occurs is correlated with the level of cerebral activity as measured by the electroencephalograph and can be increased by electrical excitation applied locally, or at a distance to nerve pathways which have connexions with the cortex[5]. In addition, some cholinoceptive neurons have recently been identified in certain cortical areas[6]. The biochemical evidence for the existence of central cholinergic neurons has therefore received substantial support from physiological experiment and there seems little reason to doubt that they form an important part of the central nervous system.

There is, however, one other cause for uncertainty in interpreting the biochemical data. This is the possibility, which has never been completely excluded, that some neuroglia may participate in ACh metabolism. As the presence of ChAc in some non-nervous tissues shows[7], ACh probably has some biological function other than that of neuro-transmitter and this could be true of the central nervous system as well as of other organs. There is no question that all neuroglial cells can be concerned in ACh synthesis since there are many parts of the central nervous system which are nearly devoid of ChAc yet contain large amounts of glial tissue. Moreover, we find that certain fractions, obtained by low-speed centrifugation from homogenized brain tissue in which glial tissue is concentrated, have an extremely low specific activity of the enzyme. So far as can be judged, therefore, this tissue does not participate to a significant extent in the production of ACh in the central nervous system. Nevertheless, as will be appreciated, there are several kinds of neuroglia, and we cannot as yet be certain that the conclusion applies with equal force to all such tissue; and so, until more evidence is available on this point, some measure of uncertainty must remain in deciding whether the regional distribution of ChAc is related solely to the distribution of cholinergic neurons.

Although there may be uncertainty in the positive identification of cholinergic neurons on biochemical data alone, there can be little or no doubt that if, as in the optic nerve and dorsal roots of most species, there is no measurable ACh or ChAc, such nerves cannot be cholinergic. It was on the basis of this kind of information that I concluded earlier that, at some central synapses, some transmitter other than ACh must be employed[2]; and, contrary to the suggestion made by Cavanagh and Holland, this conclusion applies to the hen as well as to mammals, because in this species the optic nerve has virtually no ChAc, synthesizing less than 0·1 per cent of the amount synthesized by puro cholinergic nerves. Moreover, in one experiment on a chicken in which I attempted the dissection of its spinal roots I found that the pooled dorsal roots synthesized only 4 per cent of the amount of ACh synthesized by the pooled ventral roots. This small amount of activity could be accounted for by contamination of dorsal with ventral root tissue that had occurred in the course of the dissection; but even without this allowance the great difference in level of ChAc shows that a large proportion of the sensory fibres must be without enzyme and therefore non-cholinergic.

Thus it seems reasonable to conclude that non-cholinergic as well as cholinergic neurons contribute to the nervous system of both birds and mammals.

Where there may be a difference between the two is in the relative numbers of cholinergic neurons, since the concentrations of both AChE and ChAc appear to be much higher in the chicken than in mammals. In the cerebellum of the hen, for example, the ChAc activity is about eight times greater than the highest activity obtained from the cerebellum of any mammal. Hence it is probable that relatively more cholinergic neurons contribute to the chicken cerebellum.

It is of interest to consider this difference in relation to the comparative morphology of the cerebellum. The virtual absence of ChAc from the outer layers of the cortex in many mammals indicates that few or none of its constituent neurons are cholinergic. A similar conclusion can also be drawn from the AChE distribution, as Cavanagh and Holland stress[1]. From our own evidence (Hebb, C. O., and Mitchell, J. F., unpublished work) based chiefly on ChAc measurements, it appears likely that the second-order sensory neuron from which the mossy fibres are derived are cholinergic and that these fibres supply most if not all of the enzyme in the white matter of the cerebellum. Thus, the concentration of ChAc would be expected to diminish as the cerebellum increases in size and complexity, largely owing to the addition of pathways which are not cholinergic connecting the cortex with other parts of the brain: the relatively high concentration of ChAc in the hen cerebellum could then be attributed to its simpler structure and the relatively low numbers of non-cholinergic fibres in its white matter.

The alternative explanation, that the cortical neurons in the hen cerebellum are cholinergic, seems less likely. It is suggested by the presence of AChE in some of the cortical neurons, the Purkinje cells in particular; but this argument loses force in view of the fact that in the pigeon cerebellum, which also has a high ChAc activity (about five times higher than in mammals), the cortical neurons appear to be devoid of AChE. Another point to be considered is that the main cell groups of the cerebellar cortex seem to be homologous with one another in birds and mammals and in other vertebrates. In view of this, it becomes less probable that they would alter in the specific chemical attributes that determine which transmitter substance they form, since from our knowledge of the peripheral and autonomic nervous systems the opposite conclusion may be drawn, namely, that anatomically homologous neurons are chemically homologous as well.

Finally, I think it should be noted that the evidence provided by ChAc measurements does not support the idea that cholinergic neurons are in general of less importance in higher as compared with lower vertebrates. Certainly the concentration of ChAc in the brain as a whole is greater in teleost fish and birds than in mammals, but in other vertebrates, the snake (*Natrix natrix*), the frog and the dogfish it is much lower. Within the mammalian group the enlargement of the cerebral cortex seems to have involved the addition of relatively more non-cholinergic than cholinergic neurons, but even in man it appears that cholinergic neurons form an important part of the cerebral cortex and in subcortical structures they have the same importance as in other members of the group.

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BLOOD AND SERUM GROUPS IN REINDEER COMPARED WITH THOSE IN CATTLE

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DURING a search for possible markers for population genetic work on the reindeer species, blood samples were investigated from 64 reindeer at the State Reindeer Research Station, Ripats, Sweden. The samples were studied for the occurrence of cellular antigens, plasma protein and hæmoglobin types.

Erythrocyte suspensions from 37 of the reindeer were tested against a battery of cattle blood-typing reagents, which detects 39 antigenic factors belonging to nine different genetic systems[1]. Only one of the antisera (anti-*J*) gave specific hæmolytic reactions with any of the reindeer samples, while two reagents, *I'* and *Y'*, gave rise to some weak and probably unspecific cross-reactions. The remaining samples were therefore only tested against anti-*J*.

Of the 64 reindeer, 20 (31 per cent) were found to have cellular *J*. Twenty-four calves, approximately 2 months old, were included in the investigation, and of these, 8 were found to have cellular *J*. In the reindeer then, the *J* substance seems to be developed on the erythrocytes at least at two months of age.

Anti-*J* occupies a peculiar position among the cattle blood-typing reagents. It is obtained from normal sera and it reacts specifically with cells of types *A* and *AB* in man and with sheep *R* cells[2,3]. The *J* substance, which behaves as a dominant genetic character, is primarily a soluble serum substance acquired by the erythrocytes after the birth of the animal[3,4]. The concentration of the *J* substance in the serum and/or on the cells is, however, subject to considerable individual variation[4,5].

In order to study the presence of soluble *J* substance in reindeer serum, inhibition experiments were carried out according to standard techniques[4]. Sera from nine individuals were examined. Three animals had cellular *J*, while the remaining ones had no detectable *J* on their erythrocytes, neither had they anti-*J* in their sera. One of our ordinary cattle *J* reagents was used as anti-serum, and the reindeer sera (in serial doubling dilutions) were used as inhibitors. The inhibition tests were carried out in duplicates with *J*-positive reindeer and cattle erythrocytes as test cells. The results (summarized in Table 1) show that the reindeer sera from animals having cellular *J* contain a soluble substance with the ability to inhibit the reaction between cattle

Table 1. Ability of Reindeer Sera to inhibit the Hæmolytic Reaction between *J*-positive Cells and an Anti-*J* Cattle Serum diluted 1/16 (approx. 4 Units of Hæmolysin)

Inhibitant	Highest dilution of the inhibitant causing inhibition	
	Cattle test cells	Reindeer test cell
Sera from 3 reindeer with cellular *J*	1/2–1/16	1/16–1/32
Sera from 6 reindeer lacking cellular *J*	No inhibition	No inhibition (3 cases) 1/4–1/8 (3 cases)

anti-*J* sera and cattle or reindeer cells of type *J*. Three of the sera from individuals lacking *J* on their erythrocytes also inhibited the reaction between anti-*J* and reindeer *J* cells. However, these three sera gave no definite inhibition when cattle cells were used as test erythrocytes. The remaining three sera lacked inhibiting power entirely.

Finally, a serum sample from each of the 64 reindeer was tested against erythrocytes from 30 reindeer and 10 cattle. None of the 20 sera from animals with cellular *J* substance contained any antibodies. However, 19 of the remaining sera contained antibodies against two or more of the red cell samples of type *J*. One of these sera (No. 5/55) was particularly strong. In tests against all the reindeer samples and 90 cattle taken at random, the reactions of this serum exactly paralleled those of the cattle anti-*J* serum, which is in routine use in our laboratory. The results from absorption experiments with the anti-*J* cattle serum and the reindeer serum No. 5/55 are given in Table 2. As seen from the table, the *J*-positive cells from the reindeer species absorbed out the antibodies for the *J* cells in the cattle species and vice versa.

Table 2. Reactivity of Anti-*J* Sera of Cattle and Reindeer Origin

Test cells	Cattle anti-*J* (No. 451)			Reindeer anti-*J* (No. 5/55)	
	Un-absorbed	Absorbed with cattle *J* cells	Absorbed with reindeer *J* cells	Un-absorbed	Absorbed with cattle *J* cells
Reindeer					
J-pos.	+	–	–	+	–
J-neg.	–	–	–	–	–
Cattle					
J-pos.	+	–	–	+	–
J-neg.	–	–	–	–	–

It can be concluded that the antigenic system in the reindeer species described here is remarkably similar to the *J* system of cattle and therefore the symbol *J* has been given to it. Reindeer with cellular *J* also have soluble serum *J*. However, as in cattle, soluble *J* substance seems to occur also in sera from some reindeer lacking cellular *J*. Some reindeer sera contain normal antibodies with a specificity identical or very similar to cattle anti-*J*. It is noteworthy that the serological similarity between the *J* systems in cattle and reindeer seems to be much greater than that between the cattle *J* and sheep *R* systems.

No breeding data were studied, because the sires of the animals were unknown. However, it seems justified to assume that the genetics of the *J* system in reindeer is similar to that in cattle[4,5].

The hæmoglobin and plasma proteins were investigated by starch-gel electrophoresis. Using a continuous *tris*-buffer *p*H 8·9 (refs. 6 and 7) only one kind of hæmoglobin was found among all the 64 reindeer. It migrated a little faster than the cattle hæmoglobin *A*.

The plasma proteins were analysed both with the continuous *tris*-buffer[6,7] and with a discontinuous buffer system[8]. Six different types were observed regardless of the method used. Fig. 1 shows the six reindeer types compared with a bovine transferrin

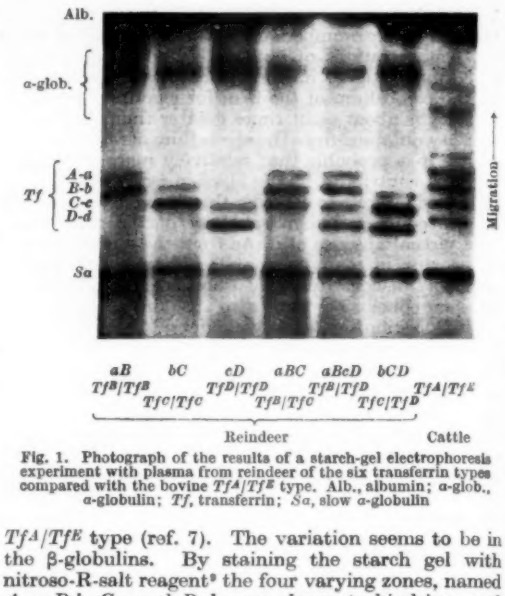

Fig. 1. Photograph of the results of a starch-gel electrophoresis experiment with plasma from reindeer of the six transferrin types compared with the bovine Tf^A/Tf^E type. Alb., albumin; α-glob., α-globulin; *Tf*, transferrin; *Sa*, slow α-globulin

Tf^A/Tf^E type (ref. 7). The variation seems to be in the β-globulins. By staining the starch gel with nitroso-R-salt reagent[9] the four varying zones, named *A-a*, *B-b*, *C-c* and *D-d*, were shown to bind iron and were therefore classified as transferrins. Capital letters are used for the intensely stained zones and small letters for the less intensely stained zones. The six transferrin phenotypes can be designated *aB*, *bC*, *cD aBC*, *aBcD* and *bCD*.

A hypothesis is proposed that the transferrin types are determined by three alleles, called Tf^B, Tf^C and Tf^D. Each allele gives rise to two transferrin fractions with a certain migration-rate in starch-gel electrophoresis. The 64 reindeer are distributed in the following way with the phenotypes in parenthesis: 6 Tf^B/Tf^B (*aB*), 18 Tf^C/Tf^C (*bC*), 4 Tf^D/Tf^D (*cD*), 15 Tf^B/Tf^C (*aBC*), 12 Tf^B/Tf^D (*aBcD*), and 9 Tf^C/Tf^D (*bCD*).

Among the reindeer tested there were 46 mother-offspring groups (several mothers had more than one offspring and sometimes daughters were mothers in other groups). The sires were unknown. All offspring had a transferrin type compatible with that of their dam except in one case where a Tf^B/Tf^B daughter was assigned to a Tf^C/Tf^C mother. This may be due to an error in the breeding records and therefore need not discount the three-allele hypothesis.

A variation in the hæmoglobin-binding proteins was also observed but the results were difficult to interpret. Some animals had a hæmoglobin-binding zone migrating slightly faster than the slow α-globulin while others had such a zone just behind the slow α-globulin. However, in most animals no distinct hæmoglobin-binding zone appeared.

We are much indebted to Dr. M. Nordkvist, of the State Veterinary Institute, Stockholm, who collected the samples of reindeer blood.

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ADENOSINE DIPHOSPHATE IN RED CELLS AS A FACTOR IN THE ADHESIVENESS OF HUMAN BLOOD PLATELETS

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THE initial reaction of hæmostasis[1] and arterial thrombosis[2–4] is adhesion and aggregation of blood platelets to the wound surface and to the site of intimal damage. The elucidation of the mechanism of this reaction is of great importance not only for diagnosis and management of hæmorrhagic disorders, but also for the problem of thrombosis. Several factors are involved: (1) a property of the platelets which enables them to become adhesive when exposed to the physiological adhesiveness-producing mechanism (thrombasthenia represents by definition the congenital lack of this property); (2) a tissue substrate for the platelets to adhere to, probably collagen and elastic fibres[5,6]; (3) a plasma factor, which is lacking in von Willebrand's disease[7] and (4) calcium. A fifth factor, possibly triggering the adhesion–aggregation reaction, was reported by Hellem as being an acidic, dialysable and heat-stable factor which was found in red blood cells[8]. This factor produced strong agglutination of platelets in citrated or oxalated plasma and converted the non-adhesive circulating platelets to adhesive platelets showing immediate adherence to foreign surfaces.

This article describes the identification of this factor as adenosine diphosphate. The effect of a number of other nucleotides on the adhesiveness of human platelets has also been examined.

The semi-quantitative test for the adhesiveness of human blood platelets described by Hellem[8,9] was used throughout this work. 0·05 ml. of saline containing the material to be tested was added to 0·5 ml. of platelet-rich plasma, and the reaction considered positive if clumping of the platelets occurred.

An extract obtained by treating human red blood cells with boiling water[8] was passed through a 'Dowex 1 × 8' column in the formate form. The column was eluted stepwise with increasing concentration of formic acid and ammonium formate–formic acid mixtures. A typical elution diagram of ultra-violet absorbing material recorded at 260 mμ is seen in Fig. 1. The eluted fractions (I–VI) were concentrated to dryness in vacuum in the frozen state, and then dissolved in a volume of saline equal to that of the original extract. Only fractions V and VI were positive in the platelet test. Occasionally the elution diagram would contain a large inactive peak at the site of fraction IV.

Fractions V and VI both yielded adenine on hydrolysis with *N* hydrochloric acid for 1 hr. at 100° C. as shown by paper chromatography in isopropanol/concentrated hydrochloric acid/water (68 : 17·6 : 14·4).

In electrophoresis at *p*H 5·7 in 0·2 *M* ammonium acetate at 900 V. for 2 hr., fraction V gave two ultra-violet absorbing zones, one of which had the same mobility and ultra-violet absorption spectrum in 0·1 *N* hydrochloric acid and in 0·1 *N* sodium hydroxide as adenosine diphosphate. By chromatography of fraction VI in isobutyric acid/concentrated ammonia/water *p*H 3·7 (661 : 10 : 329), three spots which corresponded to adenosine monophosphate, adenosine diphosphate and adenosine triphosphate were found.

The presence of adenosine diphosphate in fractions V and VI was definitely proved by a specific enzymic test[10] for adenosine diphosphate using reduced diphosphopyridine nucleotide, phosphoenol pyruvate phosphokinase, and lactic acid dehydrogenase. (Reagents for testing adenosine diphosphate were purchased from C. F. Boehringer and Soehne G.m.b.H., Mannheim.) The total change in the ultra-violet absorption at 340 mμ was used in calculating the amount of adenosine diphosphate present. In fractions V and VI, assuming all ultra-violet absorption at 260 mμ in 0·1 *N* hydrochloric acid to be adenosine diphosphate, 86 per cent and 50 per cent respectively were present according to the enzymic test for adenosine diphosphate.

Thus adenosine diphosphate was present in both the active fractions V and VI and proved to be the substance responsible for effecting the adhesiveness of the platelets. It is active in the test solution in a concentration of 0·02 μgm./ml. A similar activity was encountered with commercial adenosine diphosphate.

The presence of adenosine diphosphate as the only active substance in fractions V and VI was suggested by the following experiment: the smallest amount of the two fractions which produced a positive reaction in the test system contained, when calculated from the amount of adenosine diphosphate present in the fraction, approximately 0·02 μgm./ml., in agreement with the amount expected.

A number of commercial nucleotides have been tested for activity. The results are shown in Table 1.

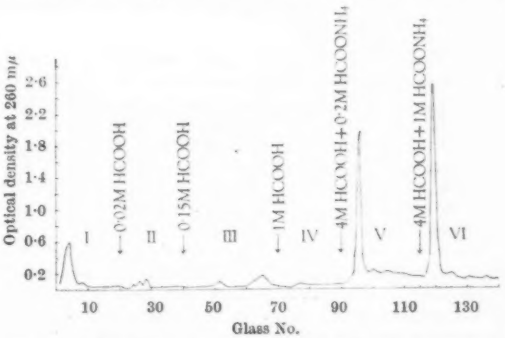

Fig. 1. Elution diagram from 'Dowex 1 × 8' (10 cm. × 1 cm.) in the formate form. 5-ml. fractions were collected. 27 mgm. of freeze-dried extract of red blood cells was dissolved in 5 ml. water and put on a column which was eluted stepwise as indicated in the diagram. The pooled fractions are indicated by I, II, III, IV, V and VI

Table 1. Effect of Unpurified Commercial Nucleotides on the Adhesiveness of Human Blood Platelets

The concentrations given for the active nucleotides represent the minimum concentration which will produce an effect

Nucleotide tested	Concentration of nucleotide in test solution (μgm./ml.)	Clumping of blood platelets
Adenosine 5′-monophosphate sodium*	100	–
Adenosine 5′-diphosphate sodium†	0·02	+
Adenosine 5′-triphosphate disodium*	2	+
Deoxyadenosine 5′-monophosphate sodium‡	100	–
Deoxyadenosine 5′-diphosphate sodium*	7	+
Inosine 5′-phosphoric acid*	100	–
Inosine 5′-diphosphate sodium§	100	–
Inosine 5′-triphosphate sodium*	100	–
Guanosine 5′-monophosphate sodium*	100	–
Guanosine 5′-diphosphate sodium§	10	+
Guanosine 5′-triphosphate sodium*	100	–
Uridine 5′-monophosphate disodium $2H_2O$§	100	–
Uridine 5′-diphosphate trisodium $2H_2O$§	100	–
Uridine 5′-triphosphate sodium*	100	–
Cytidine 5′-monophosphate sodium $2H_2O$§	100	–
Cytidine 5′-diphosphate sodium $4H_2O$§	63	+
Deoxycytidine 5′-phosphoric acid‡	100	–
Deoxycytidine 5′-triphosphate sodium*	100	–
Thymidine 5′-phosphate calcium $4H_2O$‡	100	–
Diphosphopyridine nucleotide*	100	–
Triphosphopyridine nucleotide‡	100	–
Flavin adenine dinucleotide*	310	–

* Purchased from Sigma.
† Purchased from Nutritional Biochemical Corporation.
‡ Purchased from California Corporation for Biochemical Research.
§ Purchased from Mann.

It is seen that deoxyadenosine diphosphate, guanosine diphosphate and cytidine diphosphate exhibited activity. To decide whether the activity was genuine or due to traces of adenosine diphosphate, the substances were purified by paper chromatography in the isobutyric acid–ammonia–water solvent system which will separate adenosine diphosphate from any of the three diphosphates. After purification, deoxyadenosing diphosphate was the only active material. It was active in a concentration of approximately 7 μgm./ml., whereas the other two were inactive at a concentration of 100 μgm./ml.

Adenosine triphosphate was active in a concentration of 2 μgm./ml., but was found by the enzymic test to contain approximately 1 per cent of adenosine diphosphate, which would account for the activity. The commercial adenosine triphosphate was purified by paper electrophoresis in 0·1 *M* phosphate buffer *p*H 5·7, a procedure which separates adenosine diphosphate and adenosine triphosphate. The latter substance after purification was inactive at a concentration of 84 μgm./ml., whereas the material corresponding to the mobility of adenosine diphosphate exhibited activity. It follows that the presence of adenosine triphosphate does not prevent the activity of adenosine diphosphate. Furthermore, the platelet system provides an extremely sensitive test for trace amounts of adenosine diphosphate.

The results suggest that the effect on the adhesiveness of the platelets is very specific since out of twenty-two nucleotides tested, only adenosine diphosphate and deoxyadenosine diphosphate were active, and furthermore that the 6-amino group in the purine ring and the pyrophosphate group are required for activity.

As a naturally occurring intracellular substance with the highly potent and specific effect described, it is logical to assume that the release of adenosine diphosphate by cellular damage may play an important physiological part as a trigger mechanism for the initial stages of hæmostasis and thrombosis. Investigations of the mechanism of its interaction with platelets are in progress.

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DISSECTING ANEURISMS OF THE LARGE ARTERIES OF CHICK EMBRYOS DUE TO (SWEET PEA) LATHYRISM

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PONSETI and Baird in 1952[1] succeeded in producing dissecting aortic aneurisms in young rats fed on a diet containing 50 per cent *Lathyrus odoratus*. Since that time, these vascular changes have been studied repeatedly by a number of workers. β-aminopropionitrile has been found to have similar properties to the so-called *Lathyrus* factor. By means of this compound, Lalich *et al.*[2] were able to produce dissecting aneurisms in turkeys.

There is a high incidence of aortic aneurisms due to *Lathyrus* factor in developing rat fœtuses. We were able to demonstrate this fact clearly in some of our experiments[3]. As a result of these experiments we attempted to produce vascular changes by means of fœtal chicken lathyrism.

Lathyrus odoratus seeds were finely ground, and macerated in physiological saline solution (w/w, 1 : 2) at room temperature for 2 hr. The fluid obtained by pressing this thin, watery mash was rendered free from micro-organisms by using a bacteriological filter.

Fertilized hen eggs (Leghorn white) were placed in special circular stands in a perpendicular position in an incubator, with the end of the egg containing the air space pointing upwards. On the 7th day after placing the eggs in the incubator the top of each egg was opened so that a circular hole appeared having a diameter of 1–2 cm. Each egg was covered by a glass vessel, allowing the access of air from beneath. The embryos were examined twice a day.

In our first experiment 50 eggs, treated as described, were divided into 5 equal groups. Beginning on the 7th day, we applied different amounts of *Lathyrus odoratus* extract to each egg (using the hole at the top) belonging to groups 1–4 every day. The eggs of group 5 were treated with saline solution only (0·4 ml. for one egg daily). The daily amounts of

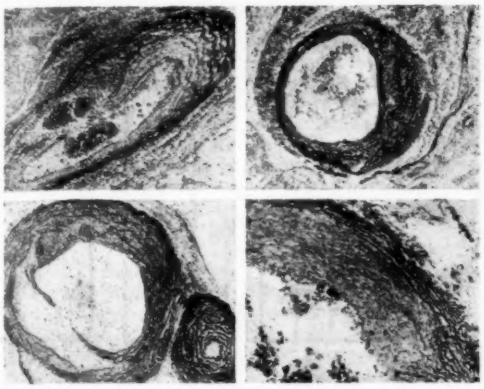

Fig. 1 (top, left). Ruptured dissecting aneurism of a branch of the pulmonary artery. (van Gieson's and Weigert's elastica stain, × *c.* 33)

Fig. 2 (top, right). Acute dissecting aneurism of ductus arteriosus. (Staining as in Fig. 1 ; × 50)

Fig. 3 (bottom, left). Acute, almost circular dissecting aneurism of the aorta. The ductus arteriosus is seen at the edge of the aorta. (Masson's trichrome and Weigert's elastica stain, × 25)

Fig. 4 (bottom, right). Scar in the aortic wall as a result of a healed dissecting aneurism. (Staining as in Fig. 3, × *c.* 83)

Lathyrus extract were as follows: Group 1, 0·05 ml./day ; group 2, 0·1 ml./day ; group 3, 0·2 ml./day ; group 4, 0·4 ml./day. In the 3rd and 4th group, the fœtuses died very early, usually during the 10th or 11th day (that is, after 2–3 days of *Lathyrus* extract application). In group 2, 6 embryos died between the 11th and 13th day, the remainder of them dying between the 18th and 20th days. In the 1st experimental group, 7 embryos died between the 15th and 20th day, and the remaining 3 hatched out on the 21st day of incubation. In the control group, 2 chickens died on the 13th day, the remaining 8 hatched spontaneously on the 20th or 21st days.

Histological examination of the experimental animals that died during the course of the experiment very often disclosed extensive hæmorrhage, especially in the thoracic region. We were able to find acute massive dissecting aneurisms affecting one of the main branches of the pulmonary artery in 3 animals, belonging to the 1st and 2nd experimental groups (dying on the 19th and 20th days). In the control group, we failed to find any pathological changes.

In the second experiment 30 eggs, treated in the same way as above, were used. They were divided into 3 equal groups on the 12th day of their development. At this time, *Lathyrus* extract was injected in a single dose by a sterile needle into the albumen at the bottom of the eggs. The hole remaining after the puncture was closed by a paraffin plug. The amount of the *Lathyrus* extract injected was as follows: group 1, 0·5 ml.; group 2, 1 ml.: group 3, 2 ml.

In this experiment, the embryos died spontaneously even up to the end of their development, that is between the 18th and 21st day. Four chickens died in the first, 5 in the second, and 9 in the third experimental group. The remaining chickens hatched out on the 20th or 21st day. At birth, they required mechanical help, as they suffered from serious impairment of leg mobility. The surviving birds were killed on the following day by ether narcosis. The thoracic organs of all the chickens were serially sectioned *in toto* for microscopic examination.

In the animals of the first group minimal vascular lesions were found. In the second experimental group we were able to find extensive dissecting aneurisms in 4 chickens: both ducti arteriosi were affected in three chicks, the pulmonary artery once, and the aorta twice. In the third experimental group, dissecting aneurisms were disclosed in six chickens, the ductus arteriosi being affected most frequently.

Several workers have studied the effects of β-aminopropionitrile on connective tissue, using chicken embryos as the experimental animal[4–6]. Roy *et al.*[7] succeeded in producing aortic ruptures by means of 2-cyanopropylamine in young chickens after birth.

The method used in our second experiment seems especially suitable for the production of vascular lesions in chick embryos by *Lathyrus* factor.

We thank Dr. Z. Rychter and Dr. L. Lemez of the Institute of Normal Anatomy, Charles University, for technical advice.

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MODIFICATION OF THE EFFECTS OF AN AMPHETAMINE–BARBITURATE MIXTURE BY THE PAST EXPERIENCE OF RATS

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AMPHETAMINE – BARBITURATE mixtures have been used in psychiatry for many years, for example in the treatment of anxiety states, and sometimes in preference to either drug on its own. Why such mixtures might be especially effective is not well understood: barbiturates in the fairly small doses used in psychiatry are usually intended to act as mild depressants or 'sedatives', while amphetamine is a cerebral stimulant and its effects on most kinds of response tend to be the opposite of those of barbiturates. Clinically, the effects of the two kinds of drug when given in combination seem to be regarded as 'mutually corrective'[1], that is, as somehow reinforcing each other's beneficial effects while mitigating each other's undesirable side actions. But relatively little systematic work on the effects of such mixtures on the behaviour of animals or man seems to have been done in the laboratory.

The purpose of the experiments described here was to study the effects of such a mixture, and of the two constituents separately, on the exploration of a new environment by laboratory rats. There is an extensive experimental literature on 'exploratory behaviour'. Many factors can affect it. There is evidence for the following generalizations[2–4]: Novel environments evoke in animals exploratory activities like walking about, rearing and sniffing which can be attributed to curiosity. Novel environments may, however, also have the opposite effect on behaviour; they may induce 'freezing' and if, for example, a noxious stimulus is introduced into a novel situation exploration is inhibited and may stop completely. These effects can be attributed to fear or anxiety. Both curiosity and fear are apt to diminish as familiarity with an environment increases, though they may do this at different rates. The actual amount of exploration in a given situation can thus be regarded as the net result of competition between two opposing tendencies; for example, radical novelty has been shown to evoke less exploratory behaviour than mild novelty, presumably because with radical novelty fear predominates. Miller[5] has shown in various experiments that the effects of fear induced in rats by electric shocks can be mitigated by barbiturates.

On this kind of analysis one might expect that drugs which can alleviate fear or anxiety would increase exploration of a radically novel environment; and they might have less effect in a more familiar environment where fear has already been largely overcome. In order to investigate this, two groups of rats were used: 'inexperienced' rats which had never been in the apparatus before, and 'experienced' rats which had been familiarized with the apparatus and had developed a relatively stable and high rate of exploring.

The apparatus was a symmetrical Y-shaped runway, 13 in. high. Each arm was 15 in. long and 5 in. wide. A trial consisted of placing a rat in the centre of the Y and leaving it in the apparatus for 3 min.; the number of times it entered the arms was the measure of exploration. Rearing and defæcation were also noted. There were no rewards. The rats were 48 adult Lister hooded females which had been reared under standard conditions since weaning and had been little handled. Throughout they lived in their home cages with food and water *ad lib.* All experiments took place between 2.30 and 5.30 p.m.

The procedure was as follows: when the rats were 117–127 days old they were allocated at random to the two experimental groups: (1) 'Experienced' rats, which were given regular trials in the runway, twice weekly, for 16 weeks. (2) 'Inexperienced' rats, which received an equivalent amount of handling but were not placed in the runway. At the end of the 16 weeks each group was divided at random into four sub-groups of 6 rats; three of each of the four sub-groups received a drug: (*a*) amylobarbitone sodium 15 mgm./kgm., (*b*) amphetamine sulphate 0·75 mgm./kgm., (*c*) these two doses combined in a mixture; the fourth sub-group were saline controls. For rats these doses are relatively small, but the proportion of barbiturate is rather higher than in the mixtures used clinically. The drugs were dissolved in saline so that the volume injected, subcutaneously, was 2 c.c./kgm. 35 min. after the injection each rat was given a single 3-min. trial in the runway.

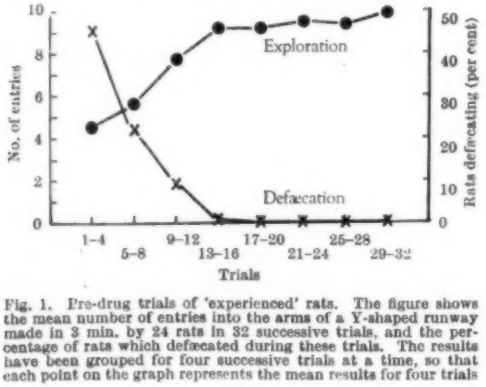

Fig. 1. Pre-drug trials of 'experienced' rats. The figure shows the mean number of entries into the arms of a Y-shaped runway made in 3 min. by 24 rats in 32 successive trials, and the percentage of rats which defæcated during these trials. The results have been grouped for four successive trials at a time, so that each point on the graph represents the mean results for four trials

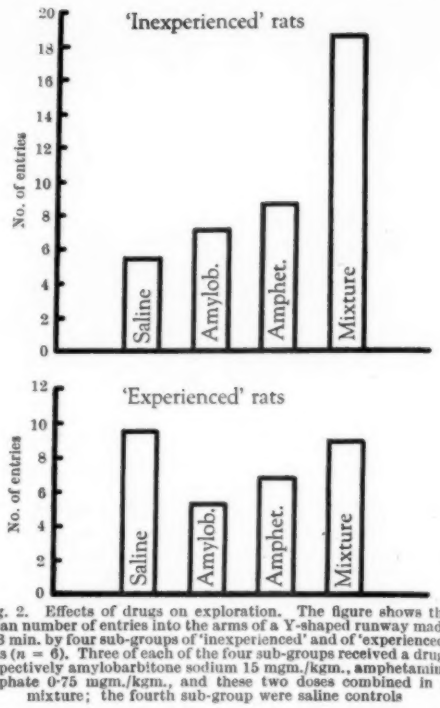

Fig. 2. Effects of drugs on exploration. The figure shows the mean number of entries into the arms of a Y-shaped runway made in 3 min. by four sub-groups of 'inexperienced' and of 'experienced' rats (n = 6). Three of each of the four sub-groups received a drug, respectively amylobarbitone sodium 15 mgm./kgm., amphetamine sulphate 0·75 mgm./kgm., and these two doses combined in a mixture; the fourth sub-group were saline controls

Pre-drug trials of 'experienced' rats. Fig. 1 summarizes the behaviour of the 'experienced' rats during the 32 pre-drug trials. At the first trial they explored relatively little (mean number of entries 5·79, *S.D.* 3·26), but in successive trials the amount of exploration gradually increased. From the 13th trial onwards the mean number of entries differed significantly from the first trial ($P < 0.02$) and soon remained fairly stable at about double the initial amount. Defæcation showed a corresponding decrease.

Effects of drugs. These are summarized in Fig. 2. The statistical significance of differences between sub-group means has been tested against the residual error, d.f. 40, discussed under (iii).

(i) 'Inexperienced' rats. The saline animals explored relatively little and spent much time sitting and 'freezing'. Their mean number of entries was 5·67, *S.D.* 2·25, which was similar to the mean number made by the experienced rats on their very first trial. Amylobarbitone and amphetamine barely increased exploration. The mixture, however, had a marked

effect: the mean number of entries was 18·83, *S.D.* 6·24, and this was more than three times the amount with saline ($t = 6{\cdot}11$, $P < 0{\cdot}001$). It was also noticed that the mixture induced unsteadiness of movement so that the rats had a rolling gait and sometimes toppled over, and some rats dragged themselves along on their bellies; in spite of this they continued to explore. Two of the amylobarbitone rats also showed some inco-ordination. It was further noticed that mixture rats were usually asleep in their home cages before being taken out for testing, and it was only in the new environment that they became active.

(ii) 'Experienced' rats. The experienced saline rats made a mean of 9·50 entries, *S.D.* 2·07, which was similar to their usual performance (cf., Fig. 1) and nearly twice the number made by the inexperienced saline rats. The drugs, however, had little effect on exploration. A few of the experienced rats showed motor inco-ordination with the mixture and with amylobarbitone, like rats in the corresponding inexperienced groups, but they explored no more than the experienced saline controls.

(iii) Interaction between 'experience' and 'drug' conditions. A two-way classification analysis of variance was carried out on the results for the four inexperienced and the four experienced sub-groups, and showed that the interaction between 'experience' and 'drug' conditions is highly significant ($F = 6{\cdot}64$; d.f. 3, 40; $P < 0{\cdot}001$). From Fig. 2 it is clear that the interaction arises in this way: among inexperienced rats the lowest mean was for saline controls while the highest mean was for the rats which had the mixture; the effect of 'experience' as compared with 'no experience' was to increase exploration under saline, but to inhibit the increase which the drug mixture induced in the inexperienced group.

These results suggest the following conclusions:

(1) Doses of amphetamine and amylobarbitone which separately barely affect exploratory behaviour can, when given in combination, have striking effects. These effects, moreover, seem to be in the same direction, that is the two drugs seem to act together to produce an increase in activity. Increased activity would not, of course, be surprising with amphetamine, but it is less usual with barbiturates. However, clinical reports have suggested that small doses of barbiturates, as of alcohol, can sometimes increase activity and excitement, and recently there have been some fresh and quantitative demonstrations of this kind of effect in laboratory animals[6–8].

(2) Whether an increase in exploratory activity occurs depends, however, not only on the drugs but also on the past experience of the animals: only rats which were unfamiliar with the new environment showed the increase. This is consistent with the assumption discussed at the outset (cf. ref. 9) that curiosity and fear may affect exploration in opposite ways and that the effect of the drug mixture should be to counteract fear: (i) Inexperienced saline rats, for whom the runway was a radically novel environment, explored less than experienced saline rats which had presumably already overcome their fear. That the experienced rats had indeed overcome their fear is also suggested by the gradual increase in exploration during the first 12 trials and by the corresponding decrease in defæcation. Defæcation has often been shown to be associated with fear and inversely related to activity (for example, ref. 10). Hence, by the time they were given drugs these rats were exploring at a rate consistent with a level of curiosity which had been stabilized by 32 previous trials, and so the drug mixture did not further increase it. (ii) But inexperienced rats given the mixture explored more than inexperienced saline rats; in other words, the drug mixture made them behave as though they were experienced and presumably unafraid. That these mixture animals explored even more than the experienced saline rats is not surprising since both curiosity and fear may be expected to be maximal on the very first occasion an animal meets a new environment; therefore, if fear is reduced, as by a drug, curiosity should more nearly show its maximal effect.

Even if further experiments do not confirm this interpretation in terms of curiosity and fear, the present results illustrate that: (*a*) the effects of drugs may sometimes only become manifest if the experimental subjects have an appropriate past history; (*b*) 'inexperienced' animals can be more sensitive to drugs than highly trained animals. If the drug mixture had been tested only on experienced rats it would have been concluded that it had no effect on behaviour.

We wish to thank Profs. F. R. Winton, G. C. Drew and A. Summerfield and Dr. A. R. Jonckheer for discussing aspects of this work with us. The work was supported by research grant *MY*–3313 from the National Institute of Mental Health, U.S. Public Health Service.

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BIOCHEMICAL ASPECTS OF PHAGOCYTOSIS

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FOLLOWING a number of observations which indicated an increased rate of oxygen consumption by leucocytes during phagocytosis, Karnovsky and his colleagues[1–3] have shown that in the process of engulfment of particles by polymorphonuclear leucocytes, obtained from peritoneal exudates of guinea pigs, changes take place in the metabolism of the leucocytes. Increases in the rates of oxygen consumption and glucose utilization occur. The most significant change is the stimulation, during the phagocytic process, of the hexosemonophosphate shunt pathway of glucose oxidation. The ratio of

glucose carbon 1 to glucose carbon 6 appearing in the respiratory carbon dioxide rises from a value of 8 in the resting (non-phagocytizing) leucocytes to a value of 22 during phagocytosis. Glycogen breakdown is increased[1-4] and there is some increase in lactate formation even though the rate of glycolysis in the leucocytes, both under aerobic and anaerobic conditions, is relatively high compared with that in many other mammalian cells. The energy for the phagocytic process seems to come from glycolysis[1]. Phagocytosis will take place under anaerobic conditions, or under aerobic conditions in the presence of a concentration of potassium cyanide that diminishes the respiration of leucocytes by 70 per cent (ref. 1). Acetate-1-^{14}C, uniformly labelled glucose-^{14}C, and phosphate-^{32}P are incorporated into the lipids of phagocytizing cells to a greater extent than into the lipids of resting cells[2,5], both under aerobic and anaerobic conditions. Incorporation of phosphate-^{32}P into phosphatidic acids is greatly increased as a result of phagocytosis[6].

The purpose of this communication is to report on certain other biochemical aspects of phagocytosis, particularly the stimulation of formate oxidation[7] and the possible bearing of this phenomenon on the mechanism by which oxygen consumption during phagocytosis is stimulated and its implications in the bactericidal processes accompanying phagocytosis.

The preparation of polymorphonuclear leucocytes was carried out by the method of Karnovsky *et al.*[1,8] with a few modifications. Guinea pigs weighing 400–500 gm. were injected intraperitoneally with 20 ml. 12 per cent sterile sodium caseinate solution. The largest numbers of leucocytes appear about 48 hr. after injection of the casein. In the following experiments, after 30 hr., the peritoneal exudates were collected in ice-cold calcium-free Krebs–Ringer medium containing 0·25 per cent sodium citrate, shaken and allowed to stand for 10 min. The cell suspension was slowly decanted from debris, centrifuged at 3,000 r.p.m. for 2 min., washed twice by resuspending in 8–12 volumes of ice-cold calcium-free Krebs–Ringer medium (*p*H 7·4). The packed cells were then diluted with the Ringer medium in the ratio 1 : 4. There was little or no contamination with red blood cells. For the preparation of a leucocyte homogenate, the following procedure was adopted. The leucocytes, after washing with calcium-free Krebs–Ringer medium, were diluted in the same medium in the ratio of 1 : 4. They were frozen, thawed and the thick suspension was homogenized for 3 min. in a Potter–Elvehjem glass homogenizer dipped in crushed ice, and the process of freezing, thawing and homogenization repeated. The homogenate was then centrifuged at 12,000 r.p.m. for 1 min. in the Sorvall centrifuge and the supernatant decanted off for use.

Much of the work described in this communication was carried out with leucocytes to which killed *E. coli* suspensions were added to induce phagocytosis. The *E. coli* cells were grown overnight on nutrient agar, collected, washed twice with 0·9 per cent sodium chloride solution, and then suspended in the same medium. They were killed by heating in a boiling water-bath for 30 min. and then centrifuged, washed with saline and suspended in calcium-free Krebs–Ringer phosphate medium.

The phagocytosis experiments were carried out in the conventional Warburg manometric apparatus at 37°. Krebs–Ringer medium (with 1·5 m*M* calcium chloride) was used as the suspending fluid. For respiration and glycolysis measurements, sodium phosphate buffer (0·02 *M*) and sodium bicarbonate (0·025 *M*) were used respectively. The initial *p*H was 7·4. The main compartment of the manometric vessel contained the leucocyte suspension (about 15 mgm. dry weight). 0·2 ml. (about 7 mgm. dry weight) heat-killed *E. coli* cells, radioactive substrates or other additions were usually tipped in from the side-arm. The centre well contained 0·2 ml. of 20 per cent potassium hydroxide with a roll of filter paper soaked in it ; in experiments with potassium cyanide as inhibitor, the potassium hydroxide–cyanide mixture described by Umbreit *et al.*[9] was used. In aerobic experiments the gas phase was air and in anaerobic experiments purified nitrogen (or a mixture of 93 per cent nitrogen + 7 per cent carbon dioxide) was used. To free the nitrogen from traces of oxygen, it was passed in succession through alkaline pyrogallol, Fieser's solution[10] and chromous chloride, and finally washed with lead acetate and barium hydroxide solutions. Such a rigorous purification was found necessary since the system oxidizing formate was found to be sensitive to the traces of oxygen still contained in commercial 'purified' nitrogen. After equilibration of the manometric vessels for 10 min. the contents of the side-arm were tipped into the main compartment of the vessel, which was incubated for 1 hr. at 37°.

Carbon-14 dioxide produced by the oxidation of ^{14}C-labelled substrates was absorbed in potassium hydroxide in the centre well of the Warburg vessel, trapped as bicarbonate and then liberated by addition of 0·3 ml. of 30 per cent trichloracetic acid which also served to terminate the reaction. The carbon dioxide was precipitated as barium carbonate in the presence of carrier, plated on aluminium planchets, and the radioactivity assayed using a thin-window gas-flow counter. The results were corrected for background and to infinite thinness of the barium carbonate layer. In experiments with ^{14}C-labelled formate, it was found that a small amount of radioactivity was always fixed by potassium hydroxide even in the absence of leucocytes ; a correction had, therefore, to be applied in a routine manner for this.

The catalase we have used was a preparation from horse liver, kindly supplied by Dr. E. Margoliash of this Institute, and had an activity of 1,200 units/ml. (ref. 11).

Metabolism of Glucose-U-^{14}C by Guinea Pig Polymorphonuclear Leucocytes

Different particles were tested for their effects on the respiration of guinea pig polymorphonuclear leucocytes in the presence of glucose and on the rate of formation of carbon-14 dioxide when using uniformly labelled glucose. Typical results are shown in Table 1. Stimulated oxygen uptake has already been shown to take place with insoluble starch particles and polystyrene latex spherules about 1·17μ in diameter[1] and with a number of different bacteria ; for example, *Mycobacterium tuberculosis*[12], *Bacillus subtilis*[13] and *Sarcina lutea*[13]. Results in Table 1 show that the highest stimulation of formation of carbon-14 dioxide among the particles tested was obtained with heat-killed *E. coli* cells. High rates of stimulation were also obtained with polystyrene latex spherules (obtained from the Dow Chemical

Company) and heat-killed *M. tuberculosis* (B.C.G.) cells grown in a Dubos albumin medium for 7 days. It should be noted that carbon and sulphur particles stimulate leucocyte metabolism, but that freshly prepared barium sulphate and manganese dioxide particles have little or no effect under the given experimental conditions. Possibly the extra oxygen consumption found when sulphur particles are added to the leucocytes is due to some oxidation of the sulphur itself. Extracts of *E. coli* were also without effect. Addition of serum (final concentration 20 per cent) or peritoneal fluid (final concentration 10 per cent) to the suspending medium made no difference to the phagocytic stimulation due to killed *E. coli* cells.

Table 1. Effects of the Presence of Different Particles on the Oxygen Uptake and Carbon-14 Dioxide Formation from Glucose-U-^{14}C by Guinea Pig Polymorphonuclear Leucocytes

Each manometric vessel contained 4 m*M* glucose-U-^{14}C (10^5 c./min./vessel) and leucocytes (15 mgm. dry weight) *p*H 7·5; 3 ml.; 1 hr.

Particles	Amount used (mgm.)	Oxygen uptake (μl./vessel)	Carbon-14 dioxide c./min./vessel
None	—	29	450
Carbon	20	58	1,670
Polystyrene (spherules)	15	124	3,430
Sulphur (powder)	60	565	1,220
$BaSO_4$ (pptd.)	95	24	660
MnO_2 (powder, fresh)	50	13	340
B.C.G. (heat-killed)	15	105	2,850
E. coli (heat-killed)	13	155	4,620

The largest accelerations of metabolism occur with fresh leucocytes. If these cells are incubated in a nitrogen atmosphere, in the absence of glucose, before the killed *E. coli* and labelled glucose are added, marked falls in the subsequent stimulated rates of oxygen consumption and carbon-14 dioxide production occur. The latter process is affected more than the former. The metabolic rates in the resting cells are apparently unaffected by this preliminary incubation procedure. The optimum *p*H for phagocytic stimulation was found to be between *p*H 7·5 and 8·5. It is noteworthy that the velocity of oxygen consumption by leucocytes, during phagocytosis of killed *E. coli*, remains nearly constant for about 40 min. at 37°, after which the velocity falls until, after 80 min., it has the same rate as that of the resting cells. Thus the stimulating process is of relatively short duration.

There is little doubt that the cationic concentrations in the media in which the leucocytes are suspended are of importance in the phagocytic stimulation of metabolism. Some typical results, given in Table 2, show that magnesium ions must play an important part in the stimulation, and that optimal effects are obtained only when magnesium, calcium and potassium ions are present with sodium chloride.

It has been pointed out[1] that phagocytic stimulation of metabolism by starch particles is proportional to the number of particles until a maximal value is reached. This is the case with killed *E. coli*. With 17 mgm. dry weight of leucocytes, the rates of oxygen consumption and carbon-14 dioxide are increased proportionately to the amount of killed *E. coli* added until approximately 6 mgm. dry weight bacteria have been added. Little further change occurs when larger quantities of bacteria are added. Similarly the increase in oxygen consumption and carbon-14 dioxide formation during phagocytosis is proportional to the numbers of leucocytes present, a maximum being reached with approximately 15 mgm. dry weight leucocytes (for the addition of 9 mgm. dry weight bacteria). With larger quantities of leucocytes (using this amount of bacteria to induce phagocytosis) there is no increase in the phagocytic stimulation of respiration and there is a fall in the phagocytic stimulation of carbon-14 dioxide formation from labelled glucose. This is possibly due to an increased demand on the glucose for glycolysis by the increased number of leucocytes. The results show that, within certain limits, phagocytic stimulation of metabolism is proportional both to the number of dead bacteria engulfed by the leucocytes and to the number of leucocytes used. It is therefore possible that the process of phagocytic stimulation may be employed for an assay method for phagocytosis.

Table 2. Effects of Cations on Metabolism of Leucocytes in Absence and in Presence of Heat-killed *E. coli*

Glucose-U-^{14}C 4 m*M*; 120,000 c./min./vessel; leucocytes, 10 mgm. dry wt.; *p*H 7·4; 1 hr.

Leucocytes washed and incubated in	Oxygen uptake (μl./vessel) Control	Oxygen uptake (μl./vessel) Phagocytosis	Carbon-14 dioxide (c./min. vessel) Control	Carbon-14 dioxide (c./min. vessel) Phagocytosis
Krebs–Ringer medium	13	101	386	5,290
Krebs–Ringer medium No magnesium ions	12	56	329	3,640
Krebs–Ringer medium No calcium ions	8	74	264	5,070
Krebs–Ringer medium No magnesium or calcium ions	12	63	244	2,560
Sodium chloride (0·9 per cent)	9	28	286	1,800

Aerobic and Anaerobic Energy of Leucocytes

Polymorphonuclear leucocytes exhibit both Pasteur and Crabtree effects[1]. The presence of glucose depresses the respiration of leucocytes (Crabtree effect), but has no diminishing effect on the stimulated respiration during phagocytosis. Thus, in a typical result, the Q_{O_2} of resting leucocytes in the absence of glucose was 3·6, and in the presence of glucose 2·1, whereas with phagocytizing leucocytes the Q_{O_2} in the absence of glucose was 15·4 and in the presence of glucose 16·0. The Crabtree effect is evidently eliminated (or masked) by the phagocytic change of metabolism.

Examination of the relationship between aerobic and anaerobic glycolysis and the Crabtree effect in resting leucocytes shows that the rate of formation of adenosine triphosphate aerobically in the absence of added glucose is identical with that in the presence of added glucose, assuming a P/O ratio of 3, in leucocytes. This is seen in a representative result given in Table 3. This relationship is already known to hold with tumours[14]. Moreover, the adenosine triphosphate made available by anaerobic glycolysis is of the same order of magnitude as that produced

Table 3. Relation between Aerobic Glycolysis $Q_{CO_2(O_2)}$ Anaerobic Glycolysis $Q_{CO_2(N_2)}$ and Inhibition of Respiration (Crabtree effect) of Resting Polymorphonuclear Leucocytes

	No glucose added	Glucose (4 m*M*)
Q_{O_2}	4·1	2·7
$Q_{ATP} = 6Q_{O_2}$	24·6	16·2
$Q_{CO_2(O_2)}$	4·2	12·0
$Q_{ATP(O_2)} = 6Q_{O_2} + Q_{CO_2(O_2)}$	28·8	28·2
$Q_{CO_2(N_2)}$	5·5	20·5
$Q_{ATP(N_2)} = Q_{CO_2(N_2)}$	5·5	20·5

XUM

Table 4. Effects of Phagocytosis on Oxidation of Different Carbon-14-labelled Substrates

Substrates 100,000 c./min./vessel; leucocytes 10 mgm. dry wt.; 1 hr. 37°. Phagocytosis of killed *E. coli*

Labelled substrate used	Glucose (4 m*M*)	Oxygen uptake (μl./mgm. dry wt. leucocyte)		Carbon-14 dioxide (c./min./mgm. dry wt. leucocyte)	
		Control	Phagocytosis	Control	Phagocytosis
Glucose-U-^{14}C	+	2·5	11·2	40	470
Glucose-1-^{14}C	+	2·5	11·4	103	1,360
Glucose-6-^{14}C	+	2·6	11·4	8	22
DL-Lactate-1-^{14}C, 2 m*M*	−	4·4	11·3	252	253
DL-Lactate-1-^{14}C, 2 m*M*	+	2·1	13·1	154	180
Pyruvate-1-^{14}C, 2 m*M*	−	4·0	9·0	97	101
Fructose-U-^{14}C, 4 m*M*	−	2·9	8·5	22	64
Galactose-1-^{14}C, 4 m*M*	−	3·0	8·1	6	22
Glycine-2-^{14}C, 2 m*M*	−	3·6	11·2	1	5
Glycine-2-^{14}C, 2 m*M*	+	2·3	12·9	2	7
L-Leucine-1-^{14}C, 2 m*M*	−	2·8	12·2	60	28
L-Leucine-1-^{14}C, 2 m*M*	+	1·9	15·5	45	35
L-Glutamine-U-^{14}C, 4 m*M*	−	3·4	10·9	228	232
L-Glutamine-U-^{14}C, 4 m*M*	+	2·2	11·5	206	217
DL-Glutamate-1-^{14}C, 4 m*M*	−	3·9	12·2	88	85
DL-Glutamate-1-^{14}C, 4 m*M*	+	2·8	15·3	75	60
Ethanol-1-^{14}C, 1 m*M*	−	2·4	9·7	198	230
Ethanol-1-^{14}C, 1 m*M*	+	1·8	10·2	159	202
Formate-^{14}C, 2 m*M*	−	3·1	12·9	63	280
Formate-^{14}C, 2 m*M*	+	1·5	13·5	69	315

aerobically (in the presence or absence of glucose). If adenosine triphosphate, made available aerobically or anaerobically, is an essential factor for the operation of the phagocytic process, it is evident that the process should take place as well anaerobically as aerobically, other conditions being the same. There is considerable evidence[1] to support this conclusion.

Evidently, since respiration is suppressed by cyanide or antimycin A (ref. 1) without interference with phagocytosis, the adenosine triphosphate produced by respiration may be localized so that it is not available for the phagocytic process.

Substrates affected by Phagocytosis

Representative results recorded in Table 4 show the effects of phagocytosis on the oxygen consumption and carbon-14 dioxide formation from a number of labelled substrates in the presence of guinea pig polymorphonuclear leucocytes and killed *E. coli*. In confirmation of earlier findings[1], it will be noted that the rate of formation of carbon-14 dioxide from glucose-1-^{14}C greatly exceeds that from glucose-6-^{14}C, justifying the conclusion that the hexosemonophosphate shunt is greatly stimulated by the phagocytic process. Only quantitatively minor effects are seen with other substrates, with the exception of labelled formate, the rate of oxidation of which is greatly increased during phagocytosis.

Aerobic and Anaerobic Oxidations by Resting and Phagocytizing Polymorphonuclear Leucocytes

During aerobic phagocytosis, the rate of oxidation of formate, as estimated by the increase of rate of formation of carbon-14 dioxide from formate-^{14}C is increased 3–6 fold. Typical results recording the quantities of substrate (glucose or formate) oxidized by 1 mgm. dry weight leucocytes per hr. are shown in Table 5. Although the oxidation of formate is markedly stimulated, there is little or no increase (within the experimental error) in the rate of oxygen consumption during its oxidation. The stimulation of oxidation of formate by phagocytosis is considerably less than that of oxidation of glucose.

Under strictly anaerobic conditions, the rates of oxidations of glucose and formate are very greatly reduced and the phagocytic process, anaerobically, brings about only relatively small increases of these rates (Table 5). However, the addition of 0·3 per cent potassium ferricyanide, well known as an oxidant of reduced phosphopyridinenucleotide[15], to the leucocytes under strictly anaerobic conditions brings about an increased rate of formation of carbon-14 dioxide from glucose-U-^{14}C with the resting cells and a large stimulation of this rate with the phagocytizing cells. The ratio of formation of carbon-14 dioxide from glucose-U-^{14}C by phagocytizing cells to that of resting cells is 9·2 in the presence of oxygen and 5·4 in presence of ferricyanide anaerobically. The rate of carbon-14 dioxide formation, however, aerobically from glucose-U-^{14}C is at least twice that obtained anaerobically in presence of ferricyanide, with both resting cells and phagocytizing cells (Table 5). It is evident that, whereas ferricyanide can replace oxygen as an oxidant in the phagocytic processes, it is, under the given experimental conditions, much less effective. What is also evident from the results given in Table 5 is that there is no stimulation, within experimental error, of formate oxidation by ferricyanide under anaerobic conditions.

Experiments carried out with sodium pyruvate show that the addition of this substance to the leucocytes under strictly anaerobic conditions increases

Table 5. Oxidation of Glucose and of Sodium Formate by Guinea Pig Polymorphonuclear Leucocytes Resting and Phagocytizing, under Aerobic and Anaerobic Conditions

Gaseous phase	Substrates	Micromoles of substrate oxidized per mgm. dry weight leucocytes per hour × 10^{1}	
		Resting	Phagocytizing
Air	Glucose-U-^{14}C	38	351
	Formate-^{14}C	24	96
Nitrogen	Glucose-U-^{14}C	5	12
	Glucose-U-^{14}C + potassium ferricyanide (0·3 per cent)	15	81
	Glucose-U-^{14}C + sodium pyruvate (5 m*M*)	32	37
	+ sodium pyruvate (10 m*M*)	27	35
	Formate-^{14}C	1	2
	Formate-^{14}C + potassium ferricyanide (0·3 per cent)	0·5	1·2
	Formate-^{14}C + sodium pyruvate (5 m*M*)	0·5	1·3

Each manometric vessel contained 15 mgm. dry weight leucocytes, 0·6 ml. Ringer medium (5 times normal strength), 0·5 ml. 0·1 *M* phosphate buffer solution *p*H 7·4, 15 micromoles glucose. Final vol. = 3 ml. Incubation = 1 hr. at 37°. Glucose-U-^{14}C, 10^{6} c./min./vessel; 2 m*M* sodium formate, 1·5 × 10^{6} c./min./vessel. Phagocytosis induced by addition of heat-killed *E. coli* suspension (7 mgm. dry weight)

the rate of formation of carbon-14 dioxide from glucose-U-^{14}C obtained with the resting cells almost to the aerobic level (Table 5). This fact lends support to the conclusion that pyruvate may be the normal oxidant for hexosemonophosphate oxidation via the shunt system[3,17]. On the other hand, the addition of pyruvate is less effective than ferricyanide in increasing the rate of formation of carbon-14 dioxide anaerobically with phagocytizing cells. Moreover, the amount of glucose-U-^{14}C converted to carbon-14 dioxide during phagocytosis aerobically is at least nine times that found on the addition of pyruvate anaerobically. It is therefore evident that whereas pyruvate can replace oxygen for glucose oxidation in resting leucocytes, it can only do so partially in phagocytizing cells. Mention should be made of the fact[21] that pyruvate can accelerate the aerobic rate of formation of carbon-14 dioxide from labelled glucose (or lactate) with ascites tumour cells, the effect being ascribed to exogenous pyruvate acting as an additional electron acceptor.

Effects of Metabolic Inhibitors and Stimulators on Glucose and Formate Metabolism during Phagocytosis

A large variety of substances has been tested for their effects on the stimulation of the rates of oxygen consumption and carbon-14 dioxide formation from glucose-U-^{14}C in presence of guinea pig polymorphonuclear leucocytes.

Antibiotics such as penicillin, streptomycin, chloromycetin, polymixin, bacitracin, gramicidin, sarcomycin were without effect at concentrations that were effective in suppressing bacterial growth.

Sodium amytal (5 mM), sodium malonate (10 mM) and sodium fluoracetate (10 mM) were without effect on the phagocytic stimulation of metabolism. On the other hand, those substances capable of suppressing glycolysis, such as phenylarsenoxide thioglycollate (30 μgm./ml.), glyceraldehyde (8 mM), phloretin (0·2 mM), as well as iodoacetate[1] and fluoride[1] bring about large inhibitions of stimulated phagocytic respiration and of carbon-14 dioxide formation from glucose-U-^{14}C. Thus the phenylarsenoxide thioglycollate brought about 87 per cent inhibition of carbon-14 dioxide formation from glucose-U-^{14}C, glyceraldehyde 72 per cent and phloretin 47 per cent. Phloretin also exercised a large inhibitory effect on the respiration of the resting cells. Again, sodium tartronate, known as a glycolytic inhibitor through its competitive suppression of lactic dehydrogenase[18], exercises a considerable inhibition of both glycolysis and phagocytic stimulation of leucocytes. At a concentration of 50 mM it inhibited glycolysis by 40 per cent and carbon-14 dioxide formation from glucose-U-^{14}C by 34 per cent in resting cells, and 68 per cent in phagocytizing cells. Sodium oxalate, another inhibitor of lactic dehydrogenase[19], behaved similarly to tartronate.

These results support the conclusion that, although the energy required for phagocytosis is obtained by glycolysis, the phagocytic stimulation of metabolism consists largely of an acceleration of the hexomonophosphate shunt pathway of oxidation and to a minor extent of an acceleration of lactic acid formation[1]. As the former process is mediated by triphosphopyridine nucleotide, it was desirable to test the effects, on the process, of substances known to stimulate oxidation of triphosphopyridine nucleotide, for example, menadione and pyruvate[16,17]. It was found that a variety of quinones, including menadione, increase markedly the rates of oxygen consumption and of carbon-14 dioxide formation from glucose-U-^{14}C of resting leucocytes with but little effect on the phagocytic stimulated rates of oxygen consumption and of carbon-14 dioxide formation. Representative results given in Table 6 show that naphthoquinone (0·3 mM) and menadione (0·3 mM) increase the rates of oxygen consumption and carbon-14 dioxide formation from glucose-U-^{14}C with resting cells several-fold, menadione being the more effective substance, while the phagocytic stimulation is only increased about 20 per cent. Vitamin K_1 and α-tocopherol quinone have little effect on the rates of oxygen consumption but increase the rates of carbon-14 dioxide formation from glucose-U-^{14}C with resting cells more than 100 per cent, and with phagocytizing cells 43 and 24 per cent respectively at the concentrations tested. It is therefore evident that quinones can stimulate the formation of carbon-14 dioxide from resting cells, sometimes to the phagocytic level, but they have much less effect on the formation of carbon-14 dioxide from phagocytizing cells. The accelerating effect of menadione on formation of carbon-14 dioxide is seen with glucose-1-^{14}C and not with glucose-6-^{14}C (Table 6), as would be

Table 6. Percentage Effects of Quinones, etc., on Metabolism of Polymorphonuclear Leucocytes

Percentage changes on metabolic rates (1 hr. incubation at 37°) only are given, phagocytosis being induced by addition of heat-killed *E. coli*

Substrate	Additions	Change in Q_{O_2}		Change in carbon-14 dioxide	
		Resting cells	Phagocytizing	Resting cells	Phagocytizing
4 mM Glucose-U-^{14}C	Naphthoquinone, 0·3 mM	+ 276	+ 22	+ 142	+ 24
	Menadione bisulphite, 0·3 mM	+ 520	+ 21	+ 565	+ 22
	Vitamin K_1, 0·5 per cent	+ 2	+ 18	+ 222	+ 43
	α-Tocopherol quinone, 0·5 per cent	− 35	− 4	+ 133	+ 24
	Phenazine methosulphate, 0·3 mM	+1240	+ 154	+1910	+ 236
	Methylene blue, 0·3 mM	+ 364	+ 15	+ 420	0
4 mM Glucose-6-^{14}C	Menadione bisulphite, 0·15 mM	+ 264	+ 30	− 3	− 18
4 mM Glucose-1-^{14}C	Menadione bisulphite, 0·15 mM	+ 330	+ 38	+1300	+ 44
,,	Phenazine methosulphate, 0·3 mM	+ 930	+ 46	+2500	+ 104
,,	Methylene blue, 0·3 mM	+ 250	+ 12	+1210	+ 12
,,	Sodium pyruvate, 8 mM	0	0	+ 74	0
,,	Sodium iodoacetate, 0·1 mM	+ 20	− 35	− 49	− 57
,,	Sodium iodoacetate, 0·1 mM + Sodium pyruvate, 8 mM	+ 14	− 24	+ 47	− 42
,,	Sodium iodoacetate, 0·2 mM	+ 5	− 48	− 64	− 63
,,	Sodium iodoacetate, 0·2 mM + Sodium pyruvate, 8 mM	0	− 33	+ 13	− 57

See Table 4 for control values.

expected if the acceleration due to menadione were confined to the hexosemonophosphate shunt.

Phenazine and methylene blue are also accelerators of the rates of oxygen consumption and carbon-14 dioxide formation in the presence of labelled glucose, their effects on the metabolism of resting cells being greater than on that of phagocytizing cells (Table 6). Phenazine is much more active than methylene blue in this respect. It is noteworthy that phenazine permits the direct oxidation of reduced diphosphopyridine nucleotide[20].

Sodium pyruvate has little or no effect on the rates of respiration of resting and phagocytizing cells; it increases (at 8 mM) the formation of carbon-14 dioxide from glucose-1-^{14}C by 74 per cent with resting cells, but has no effect on that obtained with phagocytizing cells. Its effects are very clearly seen on leucocytes treated with iodoacetate, for it nearly abolishes the inhibition of the rate of formation of carbon-14 dioxide by iodoacetate in resting cells (Table 6). On the other hand, it has a relatively small effect in diminishing the inhibition by iodoacetate of formation of carbon-14 dioxide with phagocytizing cells.

While these results indicate that pyruvate will increase the rate of formation of carbon-14 dioxide from labelled glucose with resting cells by at least 74 per cent, both with normal cells and with cells treated with iodoacetate, and that it may, therefore, act as an oxidizer of reduced triphosphopyridine nucleotide, it is clearly very ineffective as compared with a quinone such as menadione or a carrier such as phenazine. Moreover, the fact that it cannot increase the rate of oxygen consumption, or of carbon-14 dioxide formation, of resting cells to the phagocytic levels indicates that phagocytic stimulation cannot be wholly due to an increased rate of liberation of pyruvate in the leucocyte during the process of phagocytosis. It should be noted that menadione (Table 7) increases the rate of formation of carbon-14 dioxide from formate-^{14}C with resting leucocytes almost to the phagocytic level.

Table 7. Effects of Inhibitors and Stimulators on Aerobic Glucose and Formate Oxidation during Phagocytosis

Conditions as in Table 5.

Additions	$10^4 \times$ μmoles substrate oxidized/hr./mgm. leucocytes			
	Glucose-U-^{14}C		Formate-^{14}C	
	Resting	Phagocytizing	Resting	Phagocytizing
None	41	484	18	56
Flavin mononucleotide (2 mM)	52	472	37	68
Menadione (0·2 mM)	240	627	46	72
Sodium azide (1mM)	37	442	1	2
Potassium cyanide (1 mM)	36	451	6	10
Sodium iodoacetate (0·1 mM)	26	259	10	25

Evidence for Hydrogen Peroxide Formation during Phagocytosis

Experiments, typical results of which are shown in Table 7, show that the formate-oxidizing mechanism is greatly inhibited by concentrations of sodium azide and potassium cyanide that have but little effect on the oxidation of glucose, either with resting or phagocytizing cells. Iodoacetate has an inhibitory effect on formate oxidation approximately equal to that on glucose oxidation. The facts indicate that formate oxidation in the leucocyte is dependent on that of glucose, that it requires molecular oxygen as an oxidant and that it is blocked by inhibitors that affect enzymes containing hæm. As it is already known that leucocytes are rich in peroxidase[22], and as the optimum pH for phagocytic stimulation of formate oxidation is 6·0, which is the optimum for peroxidase, it is reasonable to conclude that oxidation of formate is accomplished by hydrogen peroxide formed during the oxidation of glucose by the hexosemonophosphate shunt. It is already known[23] that in the presence of peroxidase or catalase, hydrogen peroxide oxidizes formate and that azide and cyanide will inhibit the activity of verdoperoxidase.

Evidence that hydrogen peroxide is actually formed during phagocytosis comes from experiments the results of which are shown in Table 8. In the first place it is evident that the addition of catalase more than doubles the rate of oxidation of formate in the presence of resting or phagocytizing leucocytes, whereas the addition of heated catalase has no effect. Such an accelerating effect must be attributed to the action of catalase on hydrogen peroxide leaking from the leucocyte, as we have found no evidence that the catalase content of the leucocyte is increased on exposure to catalase. Secondly, the addition of sodium nitrite (2 mM) which will act as competitor to formate[23] brings about an inhibition of oxidation of formate by resting or phagocytizing leucocytes. Moreover, the system oxidizing formate is to be found in the leucocyte homogenate, which is capable of extensive oxidation of formate in the presence of a system producing hydrogen peroxide such as is afforded by a mixture of glucose oxidase (notatin) and glucose (Table 8).

Table 8. Factors affecting Aerobic Oxidation of Formate by Leucocytes and Leucocyte Homogenates

Formate-^{14}C (2 mM) present throughout. Glucose = 5 mM. Notatin = 0·01 per cent

	$10^4 \times$ μmoles formate oxidized/hr./mgm. leucocytes
Resting leucocytes	
Leucocytes only (pH = 6·0)	36
Notatin + glucose	0
Leucocytes + notatin + glucose	715
Leucocytes + notatin + glucose + sodium azide (1 mM)	1
Leucocytes only (pH = 7·4)	18
+ sodium azide (1 mM)	0·8
+ potassium cyanide (1 mM)	6
+ catalase (1/750)	40
+ heated catalase (1/750)	18
+ sodium nitrite (2 mM)	3
Phagocytizing leucocytes	
Leucocytes only (pH = 7·4)	86
+ catalase (1/750)	198
+ heated catalase (1/750)	87
+ sodium azide (1 mM)	2
+ sodium nitrite (2 mM)	12
+ potassium cyanide (1 mM)	9
Leucocyte homogenate	
Homogenate only (pH = 6·0)	65
+ glucose + notatin	529
+ glucose + notatin + catalase (1/15,000)	708
Glucose + notatin + catalase (1/15,000)	105
Homogenate + glucose + notatin + sodium azide (1 mM)	5
Homogenate + sodium azide (1 mM)	3
Homogenate + sodium nitrite (2 mM)	6

The evidence indicates that the systems oxidizing glucose (by the hexose monophosphate shunt pathway) and formate are present in the non-particulate cytoplasmic fraction of the leucocytes, a fact in agreement with observations concerning the localization of glucose-6-phosphate and 6-phosphogluconic acid dehydrogenases in many other tissues[25].

Oxidation of Sodium Sulphite by Resting and Phagocytizing Leucocytes

A substance that greatly increases the rate of oxygen uptake of leucocytes undergoing phagocytosis is sodium sulphite. In a typical experiment this substance (at 30 mM) increased the oxygen consumption per hour of 12 mgm. (dry weight) rest-

ing leucocytes from 30 µl. to 60 µl., and of phagocytizing leucocytes from 120 µl. to 700 µl. At the same time it diminished carbon-14 dioxide formation from glucose-U-^{14}C, with phagocytizing cells, by 90 per cent. Oxidation of formate-^{14}C to carbon-14 dioxide was also suppressed by sodium sulphite, an inhibition of 47 per cent occurring with 10 m*M* sodium sulphite. The reasons for these effects are now being investigated.

Participation of Flavin in the Formation of Hydrogen Peroxide in Leucocyte Homogenates

Results, which will be described in detail elsewhere (Iyer, G. Y. N., and Quastel, J. H., to be published), show that homogenates of guinea pig polymorphonuclear leucocytes are capable of oxidizing in the presence of air the reduced forms of di- and triphosphopyridine nucleotides to hydrogen peroxide, as indicated by oxidation of formate-^{14}C to carbon-14 dioxide. Reduced triphosphopyridine nucleotide is more effective than the reduced diphosphopyridine nucleotide in this respect. It seems likely that this mechanism underlies the formation of hydrogen peroxide during phagocytosis. The addition of flavin mononucleotide (0·01 m*M*) accelerates the rates of oxygen consumption of the leucocyte homogenate in presence of reduced di- or tri-phosphopyridine nucleotide. Moreover (Table 7), the addition of flavin mononucleotide increases the rate of formation of carbon-14 dioxide from formate-^{14}C in the presence of the resting leucocytes. These facts support the conclusion that a system catalysed by flavin is involved in the oxidation of the reduced di- and triphosphopyridine nucleotides by leucocytes to form hydrogen peroxide.

The liberation of a reduced di- or tri-phosphopyridine nucleotide oxidase, capable of producing with these dinucleotides, hydrogen peroxide, during the process of phagocytosis, or that of a quinone linking reduced di- or tri-phosphopyridine nucleotide with molecular oxygen, would account for the observed phenomena. Possibly the release of enzymes from the granules of polymorphonuclear leucocytes, shown by Cohn and Hirsch[26] to occur during phagocytosis, is involved in these phenomena[3,6].

Formation of Hydrogen Peroxide in Leucocytes during Phagocytosis

Calculations indicate that if the increased oxygen consumed by the guinea pig leucocytes on phagocytosis is wholly converted to hydrogen peroxide, the amount of the latter formed will amount to 0·39 micromole per mgm. dry weight leucocytes at *p*H 7·4. This corresponds to a concentration of approximately 100 m*M* in the leucocyte, assuming no leakage. If, however, the increase in oxidation of formate by the leucocytes on phagocytosis may be considered as the measure of hydrogen peroxide formation, this will amount to 1·3 m*M* at *p*H 7·4. At a lower *p*H, for example, 6·0, the corresponding values are 26 m*M* hydrogen peroxide (calculated from the increased amount of oxygen uptake) and 6·2 m*M* (calculated from the increased oxidation of formate. It is evident that the amount of hydrogen peroxide formed will vary according to the amount of phagocytosis and the intracellular *p*H and that it may vary from 1 m*M* to 100 m*M*. The latter concentration of hydrogen peroxide is known to be highly toxic to *E. coli*[27], but whether such an amount is actually formed is still uncertain. Nevertheless, in the consideration of the various factors that may operate in bringing about bactericidal action during phagocytosis, the possibility that hydrogen peroxide is formed during this process must be taken into account. It is unlikely that hydrogen peroxide is wholly responsible for the non-specific bactericidal effects of phagocytosis[28,29], but it will surely be one of the responsible agents.

Summary

Phagocytosis by guinea pig polymorphonuclear leucocytes is accompanied by an increased rate of oxidation of formate. Formate oxidation is linked with the oxidation of glucose and is sensitive to cyanide and to azide. Whereas glucose oxidation, anaerobically, may be stimulated by the presence of ferricyanide or pyruvate, formate oxidation under these conditions does not occur. The evidence indicates that whereas added pyruvate can replace oxygen as an oxidant with non-phagocytizing leucocytes, it is relatively ineffective with phagocytizing cells. The results also indicate that oxidation of reduced phosphopyridine nucleotides in leucocytes is accompanied by formation of hydrogen peroxide which is responsible for oxidation of formate. It is possible that the stimulation of glucose oxidation that occurs during phagocytosis is due to liberation of oxidase systems reacting with molecular oxygen to form hydrogen peroxide. The implications of the latter process on the non-specific bactericidal effects occurring in phagocytosis are discussed.

The award of Colombo Plan Fellowships to two of us (G. Y. N. I. and M. F. I.), under the technical assistance programme of the Canadian Department of Trade and Commerce is gratefully acknowledged.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Atmospheric Scale Height as a Modifying Influence on Linear Trend of *F*-Region Electron Density with Sunspot Number

In the prediction of maximum usable frequencies a linear increase in $foF2$ with sunspot number is generally assumed for practical purposes[1]. However, an analysis of maximum usable frequencies, estimated for a sunspot cycle interval from Christchurch ionospheric vertical sounding data obtained during 1947–58, indicated that the trend of twelve-month running average values of $foF2$ monthly medians with twelve-month running average sunspot number showed significant departures from linearity especially in the daylight hours and above running average sunspot number 170 at the 1957–58 sunspot maximum. Chun-Ming Huang[2] has observed a similar marked departure from linear trend at the 1957–58 maximum in an analysis of vertical sounding stations in the Western Pacific area, the time of onset relative to sunspot number varying with time of day and station latitude.

A critical examination of Christchurch trends in noon values of maximum $F2$ ion density ($N_{max.}$) and true height ($h_{max.}$) was made to discover possible modifying influences contributing to the observed $foF2$ departures. The plot of twelve-month running average values of $N_{max.}$ vs. running average sunspot number (Fig. 1) showed that departure from a linear trend is clearly defined for median, calm and disturbed conditions above running average sunspot number 170.

The noon maximum ion density at a fixed pressure-level can be assumed to be an approximation to the equilibrium value:

$$N = \frac{q}{\beta}$$

where q is the rate of ion production and β is the rate of loss by an attachment-like process.

On this assumption, if height changes, and changes in β throughout a cycle are neglected, an increase in q with sunspot number due to an increasing incident ionizing radiation could explain the main linear increase in $N_{max.}$ of the order of 4 : 1 for calm conditions. Height changes, however, indicative of scale height variations appear to be significant in modifying the electron density increase due to increasing q.

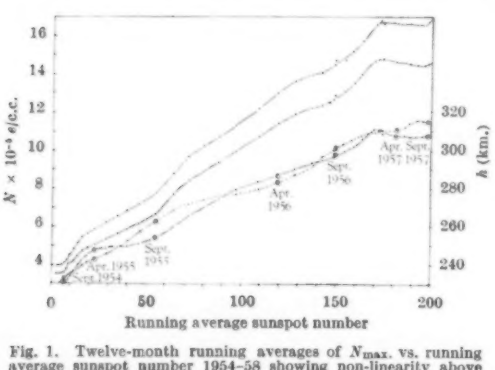

Fig. 1. Twelve-month running averages of $N_{max.}$ vs. running average sunspot number 1954–58 showing non-linearity above running average sunspot number 170 and annual residual variation. Full lines, ion densities; dotted line, median heights. Upper, middle and lower curves are for calm, medium and disturbed conditions respectively

True height values of $h_{max.}$ for representative calm days 1954–58 were available from $N'h$ profiles computed by Cummack using the method of King[3]. Where the coverage was not complete for this period a relatively quick computation of $h_{max.}$ using the median trace method[4] combined with a slider technique was used. The latter values were in reasonable agreement with the more rigorous calm-day computations and satisfactory for the purpose required.

Twelve-month running average noon values of $h_{max.}$ for the 1954–58 interval were plotted on the ion density graph and the height trend coincided with the disturbed ion density trend (Fig. 1). This latter exhibited similar departure from linearity to the median and calm ion density trends and was therefore taken as the ion density standard of reference for purposes of comparison.

Above running average sunspot number 170 the height trend as shown in Fig. 1 was maintained for a time after the linear increase in ion density had degenerated.

Assuming that (1), height increases are indicative of increased scale height (H), and (2), that $qH \propto I$, where I is the incident ionizing radiation, it is postulated that critical increases in scale height near the 1957–58 sunspot maximum have been large enough to modify the increase in q resulting in a relative decrease in $N_{max.}$ at this stage of the cycle. As the ion density departures were similar under both calm and disturbed conditions, the effect of increased incidence of storms near sunspot maximum is not considered to be a major contributing factor to this phenomenon.

Fig. 1 also shows that a residual annual variation remains during the years 1954–56 after the main seasonal variation has been eliminated by the smoothing process. Relative ion density maxima and height minima centre on April while corresponding ion density minima and height maxima centre on September. It can be considered that twelve-month running averages for April and September include the same equinoctial and winter months April–October. In addition, the April average includes the effect of the preceding summer months and the September average the effect of the following summer months. As a result of the smoothing process, therefore, any marked departures from linearity occurring in the trend due to the effect of modifying influences in succeeding summer seasons could be at a maximum at the April and September points on the graph. The observed residual annual departures then can imply that summers nearer sunspot maximum are characterized by a relative decrease in $N_{max.}$ associated with increase in scale height. This deduction was supported in an examination of the declining phase of the cycle where height and ion density departures for April and September were found to be reversed, again implying that summers nearer sunspot maxi-

mum show a greater relative decrease in $N_{max.}$ due to relatively increased scale heights than those nearer sunspot minimum.

As the increase in summer heights from sunspot minimum to maximum (240–350 km.) is much greater than the increase at other seasons (230–280 km. in winter) a modifying influence due to increasing scale heights would be expected to be greater for summer as suggested. However, a more refined discussion, including the effect of changes in β, is needed to account for the detailed seasonal behaviour pattern of $N_{max.}$ observed at Christchurch throughout the sunspot cycle investigated.

A full account of the analysis of estimated values of maximum usable frequencies throughout a sunspot cycle and associated electron density trends at Christchurch will be published in the *New Zealand Journal of Geology and Geophysics*. The survey includes detailed observations of seasonal trends in $N_{max.}$ throughout the cycle and examines sunspot cycle changes in diurnal trends of ionospheric storm departures.

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PHYSICS

Triple Resonance Phenomenon in Plasmas

INTERACTIONS between electromagnetic waves and weakly ionized plasmas in the presence of a steady magnetic field have been widely investigated[1]. The Appleton–Hartree formula for the equivalent complex dielectric constant of the plasma is partially reproduced in graphical form in Figs. 1 and 2. These graphs are restricted to the case that the propagation vector and E-polarization of the electromagnetic wave are both perpendicular to the direction of a uniform steady magnetic field. With these restrictions, the relative dielectric constant can be written:

$$\varepsilon' = \frac{\varepsilon}{\varepsilon_0} = \varepsilon_r - \frac{j}{\omega\varepsilon_0} = \frac{\varepsilon_{11}^2 + \varepsilon_{12}^2}{\varepsilon_{11}} \qquad (1)$$

in which

$$\varepsilon_{11} = 1 + \frac{\omega_p^2}{j\omega}\,\frac{\nu + j\omega}{(\nu + j\omega)^2 + \omega_b^2} \qquad (2)$$

$$\varepsilon_{12} = -\frac{\omega_p^2}{j\omega}\,\frac{\omega_b}{(\nu + j\omega)^2 + \omega_b^2} \qquad (3)$$

where ω is the radian frequency of the wave, ν is the collision frequency. $\omega_b = eB/m$ is the cyclotron frequency, $\omega_p = \sqrt{\dfrac{ne^2}{m\varepsilon_0}}$ is the plasma frequency, e and m are the charge and mass of an electron. B is the strength of the steady magnetic field, n is the volume density of free electrons in the plasma, and ε_0 is the permittivity of free space. A dependence on time of $e^{j\omega t}$ has been assumed.

When $\nu = 0$, the insertion of (2) and (3) in (1) leads simply to the result that the relative dielectric

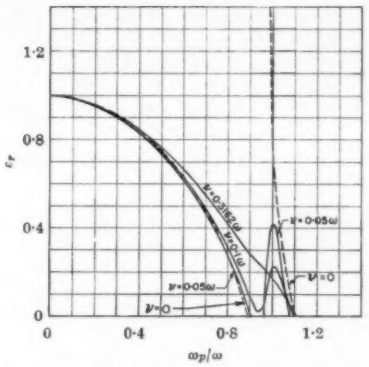

Fig. 1. Relative dielectric constant versus normalized plasma frequency. ($\omega_b = 0{\cdot}2\ \omega$)

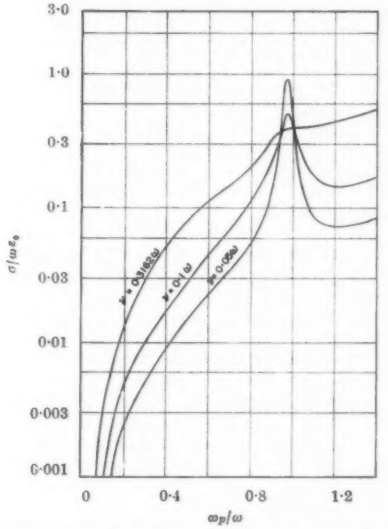

Fig. 2. Normalized conductivity versus normalized plasma frequency. ($\omega_b = 0{\cdot}2\ \omega$)

constant alternates between regions of positive and negative values as the electron density is increased. Thus the electromagnetic wave is propagated if the free electron density is less than the value corresponding to $\omega_p^2 = \omega(\omega - \omega_b)$. It is cut off in the range $\omega(\omega - \omega_b) < \omega_p^2 < (\omega + \omega_b)(\omega - \omega_b)$ and is then propagated again in the range $(\omega + \omega_b)(\omega - \omega_b) < \omega_p^2 < \omega(\omega + \omega_b)$. For all electron densities greater than the value corresponding to $\omega_p^2 = \omega(\omega + \omega_b)$ the electromagnetic wave is not propagated. It is apparent that increasing ω_b serves to separate these boundaries farther from each other. In the laboratory, it is possible to achieve a sufficiently large ω_b/ω to make this triple resonance effect detectable.

The inclusion of collision losses serves to broaden these resonances, as in any problem in which Q is lowered. However, it is interesting to note that a sufficiently high value of ν will erase the triple resonance effect entirely, as shown in the ε_r curves of Fig. 1. This proved to be a formidable stumbling block in the experimental work which was undertaken to demonstrate this phenomenon.

XUM

An apparatus similar to one used earlier by Anderson[2] was employed. A 19-mm. outside diameter 'Pyrex' tube with a 1·5 mm. wall was placed inside a square brass wave-guide. The glass tube was 42 in. long with the last 3 in. on each end tapered to a point. The inside dimensions of the wave-guide were 0·805 in. by 0·805 in. with 0·035-in. walls. 5·5-in. wave-guide transitions coupled each end of the square guide to standard *X*-band wave-guide for connexion to the remainder of the microwave system. A klystron source was operated at 8,600 mc.p.s. and unmodulated.

The tube was attached through a hole in the wave-guide wall, with a 10-mm. nipple, to a spherical bulb of 3 in. diameter. The latter contained the ignitor electrode, auxiliary anode, and mercury pool cathode from a *GL*-5779 'Ignitron'. A second hole in the wave-guide wall, at the other end of the plasma column, accommodated a 5-mm. nipple which housed the main anode, a 0·1-in. diameter tungsten rod. The rod did not project into the plasma column; its end was even with the inside wall of the main tube. Continuous pumping was used to maintain the vacuum between 10^{-6} and 10^{-7} mm. of mercury.

A transverse d.c. magnetic field was impressed on the plasma by inserting the square wave-guide between the 1-in. wide by 57-in. long poles of an electromagnet. The magnet core was constructed of 1020 cold-rolled steel. The 17-gauge heavy 'Formvar' winding was continuous around the 57-in. dimension of the magnet, giving a uniform magnetic field along the entire length of the plasma column. A 0–150 V. d.c. generator supplied a maximum of 4 amp. to the winding to give a maximum field of 1,200 gauss.

The ignitor electrode was pulsed at 60 c.p.s. from a 600 V. supply and the main anode was energized from a composite source—5 kV. to provide a d.c. current plus a variable 60 c.p.s. supply to superimpose a sinusoidal variation on the d.c. current. In this manner, the plasma properties were made to vary at a 60-c.p.s. rate with controllable excursions. The results of this experiment were recorded by photographing the traces of a dual beam oscilloscope for various values of d.c. magnetic field. A representative set of traces is reproduced in Fig. 3. In each display the upper trace is the a.c. component of tube current and the lower trace is the detected microwave signal which has traversed the plasma column. All the oscillograms shown in Fig. 3 were taken with 420 m.amp. d.c. and constant amplitude a.c. through the discharge tube. The negative peak of the upper trace corresponds to minimum electron density in the plasma, a value small enough to cause essentially full transmission of the microwave signal in the absence of a d.c. magnetic field.

The lower traces have many features in common. The 8,600-mc.p.s. wave is cut off until such time as the current through the plasma tube (and thus the electron density) lowers to the point where propagation begins; this is a resonant point. The propagation then rises to a peak, giving a second resonant point. The propagation again decreases, the minimum point giving a third resonance. The propagation then rises to a maximum value of transmission. As the tube current increases in value this sequence is repeated in reverse order.

It is evident that as the d.c. magnetic field is increased, and both the d.c. and a.c. current-levels are kept constant, the transmission-level decreases. This is attributable to the increases in electron density

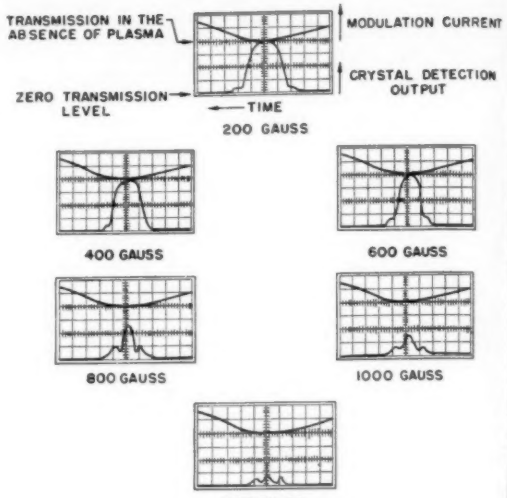

Fig. 3. Oscillograms showing the variation of modulation current and transmitted power during one de-ionization and ionization process for six different magnetic field values. (Reading from right to left, de-ionization occurs in time before ionization)

and collision frequency which accompanied the increases in the d.c. magnetic field. It is interesting that Beckman[3] has predicted just such an effect.

For this reason no attempt was made to infer that there were specific values of plasma frequency at the three resonant points. No claim can be made that the three resonances separated on the ω_p scale as ω_b was increased. However, the triple resonance phenomenon showed up very clearly and this was the principal goal of the experiment.

Considerable difficulty was experienced in obtaining the results shown in Fig. 3. It was found that the apparatus attained a considerable temperature due to heat generated in the plasma and in the winding of the d.c. magnet. This caused the collision frequency to increase to the point that the triple resonance effect was obscured as predicted by the data in Figs. 1 and 2. Indeed, when the apparatus was first turned on, the oscilloscope trace would clearly show a triple resonance, and as one watched, these features would subside until finally no resonance phenomena were displayed.

This difficulty would not necessarily be present in measurements on a plasma which was not supporting a large current. Often the collision frequency is small in problems concerning the plasma sheath around a missile, or the wake behind it, or in problems concerning the ionosphere. Such plasmas do not contain large currents and their exact laboratory stimulation must be accompanied by techniques different from the one used here. In such applications it can be expected that joint use of a d.c. magnetic field and electromagnetic waves can invoke a triple resonance effect which will prove useful in diagnostics.

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Excitation of the 2s State of Atomic Hydrogen by Electron Impact

In a recent publication Kingston, Moiseiwitsch and Skinner[1] have applied the second Born approximation, retaining only terms up to the third order in the interaction energy, to the calculation of the total cross-section Q ($1s$–$2s$) for the $1s$–$2s$ excitation of hydrogen atoms by electrons. Their results are displayed in Fig. 1. Curve 1 is the cross-section obtained by using the first Born approximation while curves 4 and 6 are respectively the cross-sections given by the second Born approximation with allowance for: (*a*) distortion due to the coupling to the $1s$ and $2s$ states; (*b*) polarization due to the coupling to the $2p$ states as well as distortion due to the $1s$ and $2s$ states.

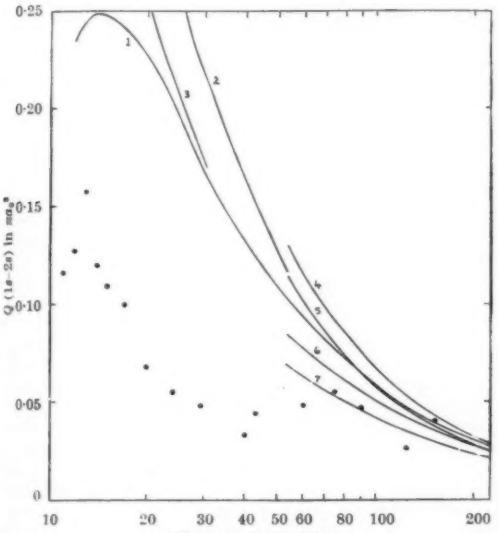

Fig. 1

Wu[2] has reported first Born approximation calculations of Q ($1s$–$2s$) in which allowance for exchange has been made. From the tables given by Wu the contribution resulting from exchange can be readily calculated and incorporated into the second Born approximation calculations of Kingston, Moiseiwitsch and Skinner. The resulting cross-sections are displayed in Fig. 1. Curve 5 includes the effects of distortion and exchange while curve 7 includes in addition the effect of polarization. Allowance for exchange reduces the excitation cross-section. Smith, Miller and Mumford[3] have carried out exact solutions of the coupled differential equations for the $1s$–$2s$ electron impact excitation of atomic hydrogen derived by making full allowance for the distortion due to the $1s$ and $2s$ states but neglecting exchange. The cross-section obtained by them is given by curve 2 and is in satisfactory accord with the second Born approximation curve 4 calculated by Kingston, Moiseiwitsch and Skinner.

Smith[4] has solved the coupled integro-differential equations for the s, p, d partial waves making full allowance for exchange as well as distortion. The resulting total cross-section Q ($1s$–$2s$) is given by curve 3, the higher-order partial cross-sections having been estimated with the first Born approximation. Curve 3 lies somewhat below curve 2, and thus allowance for exchange reduces the cross-section in agreement with the previously noted effect of exchange on the second Born approximation cross-sections.

The experimental points of Stebbings, Fite, Hummer and Brackmann[5] for Q ($1s$–$2s$) are also displayed in the figure. Lichten[6] has shown that the original data of Stebbings *et al.* are in error and should be multiplied by a factor 3/2. This correction was therefore introduced (cf. Hummer and Seaton[7]).

There is satisfactory agreement, at least at energies greater than 60 eV., between the modified experimental results of Stebbings *et al.* and the cross-section curve 7 in which allowance for exchange as well as distortion and polarization has been included. Noting that curve 4 lies slightly above curve 2, it is to be anticipated that curve 7 is slightly in excess of the actual $1s$–$2s$ excitation cross-section, which would then bring calculation into still closer agreement with the absolute experimental data of Stebbings *et al.*

The experimental cross-section obtained by Lichten and Schultz[8] does not extend above 45 eV., and in any event is not absolute. Hence no comparison between theory and their experimental results is feasible. However, their relative cross-section is in good agreement with the shape of the cross-section obtained by Stebbings *et al.* as was pointed out by the latter authors.

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Measurement of Stopping-Power Ratio for Cavity Ionization Chambers

The energy absorbed in a medium per unit mass, E_m, exposed to X- or γ-radiation can be related to the ionization produced per unit mass of gas, J_m, situated in a cavity in the medium by the Bragg–Gray equation:

$$E_m = \frac{1}{F_m} \cdot WJ_m \qquad (1)$$

where W is the average energy associated with the production of one ion pair in the gas, F_m is the mass stopping-power ratio of the gas to the solid for electrons. F_m has been evaluated theoretically by assuming electrons lose energy continuously[1,2] and also by allowing for discrete losses of energy[3,4]. However, experimental investigation has shown that these theoretical treatments are only useful for a limited range of size of cavity[5], as indeed the initial assumptions in their derivations imply. In many situations the greatest uncertainty in measuring exposure or absorbed dose arises from uncertainty in this factor. Thus a direct measurement of F_m is desirable.

Several workers have measured the relative stopping-power ratio, but Whyte[6] devised a method

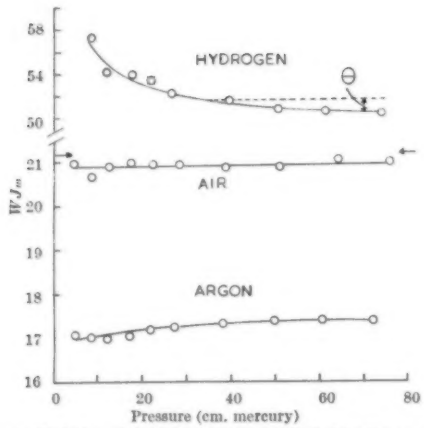

Fig. 1. Variation of the ionization/gm. multiplied by the average energy associated with the production of one ion pair with the gas pressure in a graphite chamber irradiated by cobalt-60 γ-rays. θ is the change in the ionization/gm. between 70 and 35 cm. of mercury pressure

of measuring the stopping-power ratio absolutely by varying the air pressure in a series of ionization chambers with walls of different atomic number.

An absolute measurement of the stopping-power ratio can also be made by varying the pressure of different gases introduced into a single ionization chamber in the following way: Fig. 1 shows the variation in the ionization/gm. of gas with the gas pressure for hydrogen, air and argon in a graphite ionization chamber irradiated by 1·25-MeV. photons (cobalt-60 γ-rays). The experimental technique has been described elsewhere[7]. Hydrogen with an atomic number less than graphite yields a graph with a negative gradient, while air and argon with an atomic number higher than graphite yield a graph with a positive gradient. The magnitude of the variation in the ionization/gm. with the gas pressure increases with the magnitude of the difference between the atomic number of the gas and the wall and hence increases with the factor, F_m.

To measure this variation of the ionization/gm. with the gas pressure a parameter, θ, has been chosen. θ is the change in the ionization/gm. between 70 and 35 cm. of mercury pressure. The value of θ for each gas is plotted against the value of WJ_m at 70 cm. of mercury pressure for each gas in Fig. 2. Now if it were possible to fill the cavity with a gas exactly equivalent to the graphite wall, F_m would be unity

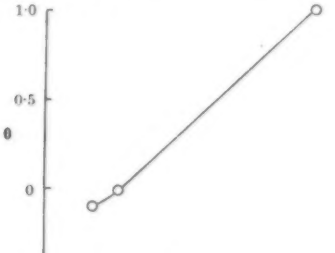

Fig. 2. Variation of the change in the ionization/gm. with the ionization/gm. multiplied by the average energy associated with the production of one ion pair

at all gas pressures. Thus the ionization/gm. would be independent of the gas pressure and in Fig. 1 a straight line parallel to the x-axis would result. In this case θ would be zero. It follows that the value of WJ_m in Fig. 2 where θ = 0 is the value that would occur when the gas in the chamber is equivalent to graphite, namely, when $F_m = 1$.

For the three gases used in this work W is known reasonably accurately and the values adopted are 36·3 eV. for hydrogen, 33·8 eV. for air and 26·1 eV. for argon. From equation (1) it may be seen that F_m is proportional to WJ_m. In Fig. 1 the ionization/unit mass of gas has been multiplied by the appropriate value of W for each gas. Thus the ordinates in Fig. 1 are proportional to the stopping-power ratio, F_m. Now the value of WJ_m when $F_m = 1$ can be determined as described here and is represented by the arrows in Fig. 1. The absolute value of the stopping-power ratio, F_m, for any of the gases in graphite at a particular pressure is given by the ratio of the ordinate of the appropriate curve at that pressure to the ordinate of the arrows, for which $F_m = 1$.

The values of the stopping-power ratio, F_m, obtained in a preliminary investigation of the method were 2·39 ± 0·05 for hydrogen, 0·99 ± 0·02 for air and 0·82 ± 0·02 for argon for a 0·5-cm.³ cavity in graphite at atmospheric pressure irradiated by 1·25 MeV. photons (cobalt-60 γ-rays). A more refined technique would considerably reduce the uncertainty quoted for these figures. In very accurate work it would probably be necessary to consider the effect of the transfer of slow electrons between the electrodes[8].

Although this method of measuring the stopping-power ratio requires data concerning W, it does offer the following advantages over Whyte's method: (1) gases are more easily interchanged than the chamber wall material; (2) there can be no errors arising from differences in volumes of the chambers as in Whyte's method; (3) to perform the interpolation in Fig. 2. it is obviously an advantage to obtain values of θ well above and well below θ = 0. Thus, for example, to determine the stopping-power ratio for a graphite chamber containing air (a combination used frequently) materials are required with an atomic number, Z, well separated from the range Z = 6–8, as well as close to this range. By interchanging the wall materials Whyte's method is limited to conducting solids and therefore can only go down to beryllium (Z = 4). However, provided the appropriate relative values of W are available, this method can use hydrogen (Z = 1) and helium (Z = 2) both well separated from Z = 6–8. Above Z = 6–8 there are several suitable materials for both methods; (4) there can be no error arising from differences in the photon attenuation in the walls or from differences in the absorption coefficient of the wall materials.

I thank Prof. J. E. Roberts for his supervision and Dr. J. R. Mallard for his encouragement.

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BIOPHYSICS

Estimation of Mass Thickness of Biological Samples from their Electron Micrographs

DURING the past several years a number of investigations[1–13] have been made to compute the mass thickness of biological samples, from the contrast in their electron microscope images. The basis of all these measurements is the well-established relation:

$$I_t/I_0 = \exp(-S.\rho t) \quad (1)$$

where I_t/I_0 is the image contrast of an elemental object area of mass thickness ρt and S is the scattering cross-section per gm. of the object. By using carbonaceous samples, for example, spheres of polystyrene latex, of known diameter and density and measuring their image contrast, several authors[3,12] have estimated the value of S under various operating conditions of the electron microscope. From these results, curve 1 in Fig. 1 has been plotted, showing the variation of S with the angular aperture α, for 50-kV. energy electron beam. A few values of S for (i) carbon film calculated from the experimental data of Lippert[14] and (ii) 'Formvar' film (from data of Bahr[5]) have also been plotted in Fig. 1.

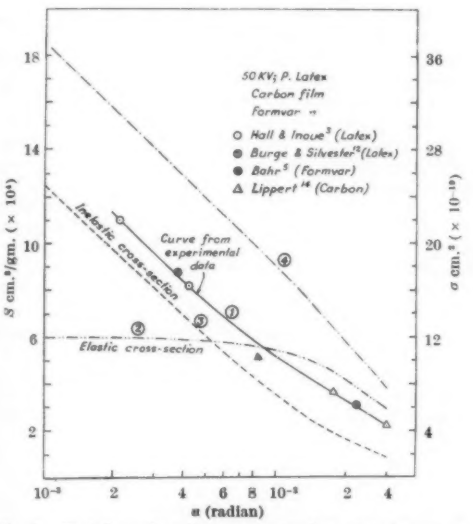

Fig. 1. Variation of scattering cross-section of carbonaceous samples for 50-kV. electron beam

Considering the samples to be composed mainly of carbon, the scattering cross-section per atom is $\sigma = AS/N_A$, that is, $2 \times 10^{-23}.S$ cm.², and the same curve (1) represents σ versus α, with suitable change of scale in the ordinate.

For comparison, the values of the elastic and inelastic cross-sections of the carbon atom, calculated from the electron scattering formula of Lenz[15] as modified by Sadhukhan[16], are shown as curves (2) and (3), while curve (4) represents the sum of the two cross-sections.

The following conclusions can be drawn from these curves. The scattering cross-section varies with angular aperture even in the range of 10^{-3}–10^{-2} radian; the nature of the variation indicates that the inelastic component of scattering seems to play an important part in the small angular region. The experimental values of the scattering cross-section are not in quite satisfactory agreement with Lenz's equations. Hence, any attempt to compute the mass thickness by making use of the theoretical electron scattering formula may give only the order of magnitude, but there is a possibility of a wide discrepancy as regards the right value.

I wish to thank Prof. N. N. Das Gupta for his kind interest in this work and Mr. P. Sadhukhan for helpful discussion. Thanks are also due to the Ministry of Scientific Research and Cultural Affairs, Government of India, for financial assistance.

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RADIOCHEMISTRY

Isolation of Radioactive Bismuth from Metallic Lead and its Compounds

IT is self-evident that the isotopic radionuclide lead-210 will remain an inseparable part of the bulk lead metal which is produced during the extraction of this metal from its various mineral ores.

This lead isotope, having a half-life of 22 years, is a direct daughter product of the radium decay chain, and is a natural precursor of bismuth-210, the latter radionuclide decaying by β-decay to the α-emitting polonium-210.

$$\underset{22\text{ years}}{^{210}\text{Pb (Ra}D\text{)}} \xrightarrow{\beta} \underset{5\text{ days}}{^{210}\text{Bi (Ra}E\text{)}} \xrightarrow{\beta} \underset{138\text{ days}}{^{210}\text{Po (Ra}F\text{)}}$$

It has been established experimentally that factual information regarding the concentration of various radioactive isotopes in normal samples of metallic lead may be obtained by the radiochemical separation and determination of the bismuth isotope. This is achieved in a quantitative manner by the acidic dissolution of 10–20 gm. of the lead material, adding approximately 10 milligrams of bismuth as carrier in the form of the nitrate, and precipitating the bismuth with an *n*-propyl gallate solution in 0·01 normal nitric acid solution at boiling point. The yellow crystalline precipitate formed consists essentially of the salt, $C_{10}H_9O_8.Bi.H_2O$ (molecular weight = 436·188), and contains 0·4790 gm. bismuth per gm. of precipitate dried at 110° C.

In experimental practice, the precipitate may be adequately dried by washing with ethanol with suction prior to weighing. During the present experiments the filtration was carried out on a

Whatman 541 filter paper of 22 mm. diameter; this being mounted on a 1-inch aluminium planchet and counted under a conventional end-window Geiger tube.

Alpha particles from any contaminating polonium-210 would be satisfactorily excluded by the Geiger window.

Extrapolation of the count-rate to zero time (time of precipitation) shows that the emission decays with the characteristic half-life of 5 days, thus positively identifying the bismuth-210 isotope. Typical experimental data are illustrated graphically in Fig. 1.

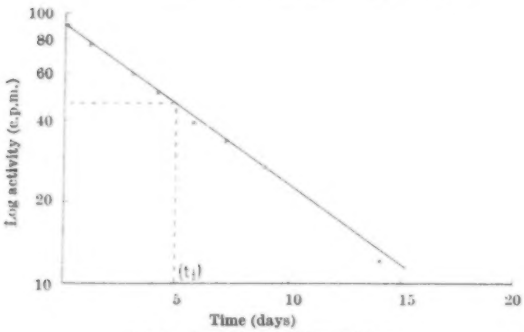

Fig. 1. Decay curve of bismuth-210

The count-rates are related on a quantitative basis with those given by a standard solution of radium *D*, *E*, *F*, from which bismuth has been precipitated in the same way. The chemical yield of the precipitated bismuth is invariably 100 per cent.

Table 1 gives some of the results of these investigations on various samples of lead and its oxides. It may be noted that sample No. 280 was carefully counted using an anti-coincidence type Geiger assembly, whereas all the other samples were done by normal Geiger tube counting.

Table 1. Radioactivity of Samples of Lead

Laboratory No.	Lead sample	Bismuth-210 β-activity μμc./gm.	Total α-activity μμc./gm.
279	Remelt ingot	3·32 ± 0·15*	4·7 ± 0·5
280	Scrap lead piping	0·17 ± 0·02	< 0·5
281	Remelt lead	1·46 ± 0·2	1·6 ± 0·6
282	Scrap window flashing	1·44 ± 0·08	2·54 ± 1·0
283	Remelted scrap	2·4 ± 0·07	5·1 ± 0·5
284	Litharge	2·08 ± 0·06	1·56 ± 0·5
286	Lead (BSS 602)	15·3 ± 0·3	16·5 ± 0·5
288	Litharge	4·2 ± 0·8	2·9 ± 0·7
291	Red lead	4·50 ± 0·15	3·8 ± 0·8
292	Litharge	13·0 ± 0·5	12·0 ± 1·0
294	Litharge	2·7 ± 0·2	2·6 ± 0·6
295	Virgin lead	2·3 ± 0·1	4·3 ± 1·0

* 90 per cent confidence limits; errors on counting only.

The order of radioactivity shown is less than the figure of 20 μμc./gm., which is the proposed limit for natural lead given in the Radioactive Substances Act, 1960 (Sec. 18, Schedule III).

W. E. Grummitt *et al.*[1], by an alternative method, quote the figures of 0·17 μμc. total β-activity per gm. for sixteenth-century lead, and 1·7 μμc./gm. for modern lead. These values are in complete accord with those obtained by the bismuth method in the present investigation.

In the final column of Table 1, experimental values are given for the total alpha activity of the individual lead samples used. These activities were determined by counting infinitely thick sources in a scintillation counter and comparing the results with those obtained from an artificially prepared lead standard containing a known amount of radium *D* in equilibrium with its daughter products. This was prepared by precipitating inactive lead sulphide in the presence of a known amount of radium *D*, *E*, *F*, and the $Z^{2/3}$ relation of J. L. Glasson[2] was used for correcting the results for the lead compounds of different average atomic number to the lead sulphide standard.

The numerical agreement between the measured alpha and beta activities in the final and penultimate columns of the table strongly suggests that the equilibrium product, polonium-210, is the main α contaminant in commercial lead samples, and that radium and other emitters can only be present in small concentrations.

The method described in this paper for the separation of bismuth radionuclides from abundant lead has many obvious applications which are now being explored in this Laboratory.

In a recent paper presented by A. Turkovitch *et al.*[3] it was shown that radioactivity induced in the lead sheath of the recovered satellite *Discoverer XVII* by solar-flare on November 12, 1960, was largely due to the production of the 15-day bismuth-205 which emits X-rays in the 70-keV. region.

Separation was carried out by the electro-deposition of bismuth carrier on a platinum electrode immersed in the lead electrolyte obtained by acid dissolution of the satellite sample (cf. A. D. R. Harrison *et al.*[4]).

It may be confidently predicted that the method now described here could be usefully employed in satellite investigations of this character, and would afford a radiochemical alternative to the electrochemical method of separation.

It may be mentioned that occasionally samples of lead are naturally contaminated with inactive bismuth, and where this occurs, the appropriate correction must be applied for the radiochemical yield.

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CHEMISTRY

Radiation Effects on Lithium-doped Nickel Oxide Catalysts in Carbon Monoxide Oxidation

Effects of nuclear radiation on heterogeneous catalysts has been the subject of various investigations in the past few years[1,2]. These investigations have shown that significant changes due to irradiation are produced in the catalytic behaviour of some oxides and metals. The phenomenon is due to the fact that catalytic activity is strongly dependent on the structure of solids and this structure may be modified on irradiation. Nuclear radiations may create in semiconductors new energetic levels, which change the position of the Fermi-level in the solid. This leads to changes in various structural sensitive properties, for example, the activation energy of the reaction catalysed by the surface of the solid[3].

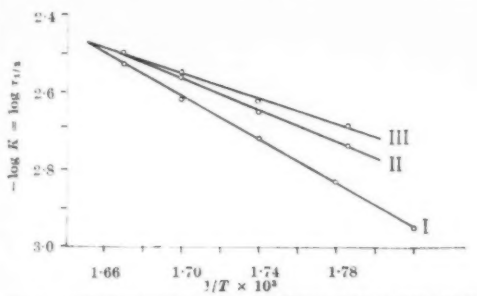

Fig. 1. Arrhenius plot for catalytic oxidation of CO on NiO + Li_2O catalyst before (I) and after (II) 80-hr. irradiation in the nuclear reactor and after one further irradiation (III) of 80 hr. Catalyst: NiO + 2·5 mol. per cent Li_2O

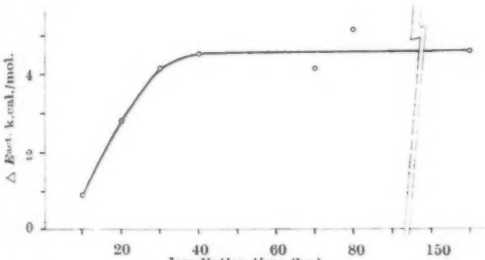

Fig. 2. Activation energy differences $\Delta E_{act.}$ (before and after irradiation) versus irradiation time

However, the major part of the cited papers deals with the variation of the reaction-rate after irradiation at a given reaction-temperature, and only in three papers are there presented results about variation of the activation energy[4–6]. In our opinion, from the changes in the activation energy following irradiation one can get more information about how irradiation affects catalytic behaviour of solids and, on the other hand, how to correlate the change of catalytic and electrical properties via activation energies.

In the present communication, we present the results of radiation effects on catalytic behaviour of solid solutions of nickel oxide (NiO) and lithium oxide (Li_2O) in carbon monoxide oxidation. The choice of NiO was determined by the fact that its electrical and catalytic properties are better known, so that it is easier to give an interpretation of the experimental results. Doping with lithium enhances the efficiency of nuclear radiations through the reaction $Li(n,\alpha)^3H$. Thus, in the neutron flux the lithium atoms become an important source of internal irradiation. On the other hand, the results of the catalytic oxidation of carbon monoxide (CO) yield more reproducible results on NiO + Li_2O than on pure NiO (ref. 7).

The effects of thermal neutron irradiation of NiO + Li_2O catalysts for the decomposition of nitrous oxide (N_2O) have been examined by Saito *et al.*[6], but this work does not indicate a clear variation of the activation energy.

Our experimental conditions were the following: irradiations were made in the thermal column of the *VVR–S* 2,000 kW. water-cooled and water-moderated enriched uranium reactor of the Institute of Atomic Physics, Bucharest. The fluxes were: thermal neutrons: 2×10^{11} *n.*/cm.² sec., and fast neutrons: 7×10^9 *n.*/cm.² sec.

The catalyst samples were obtained from the pyrolysis of $Ni(NO_3)_2$. The NiO powder was mixed with Li_2O (from Li_2CO_3), in proportion of 2·5 mol. per cent, and sintered at 850° C. for 3 hr. The oxidation of carbon monoxide was followed in a closed system[7] as indicated by Dry and Stone[8]. The results of a typical experiment are represented in Fig. 1. Fig. 2 shows the variation of the activation energy differences (before and after irradiation) versus irradiation time. From these figures, it is clear that irradiation significantly affects the activation energy of the catalytic oxidation of carbon monoxide and that the effects increase with increasing irradiation time. The experimental values from Fig. 2 seem to imply the existence of a saturation effect revealed by a plateau beginning with irradiation times of the order of 40 hr.

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Hydrogen Overvoltage at Cathodes of 77 per cent Palladium and 23 per cent Rhodium

Cathodizing an equilibrium $Pd/H/H_2$ electrode (25° C. : 1 atm. hydrogen: zero volt potential with respect to a $Pt/H/H_2$ reference in the same solution) further increases its hydrogen content (H/Pd, atomic ratio) and its corresponding hydride vapour pressure p. These increases are reflected as a component of overvoltage (volts) given[1–3], the symbols η_2 or η_d (termed[1,3] the diffusion or hydride overvoltage) and related to p by $\eta_2 = 0{\cdot}029 \log p$. The large capacity of palladium for hydrogen has enabled η_2 to be obtained by extrapolation, back to zero time, of the increase of electrode potential, E, which follows interruption of cathodization, using established relationships between E, p and H/Pd (refs. 1–3). From such measurements it has been established[1,3–5] for surfaces active for rapid equilibration with hydrogen molecules dissolved in solution that at current densities i less than 0·040 amp. cm.$^{-2}$ the retarding kinetic step maintaining H/Pd in excess of its equilibrium value (1 atm. hydrogen: 25° C.) is the slow diffusion from the cathode of hydrogen molecules formed during electrolysis. Under these conditions, η_2 is related to i by:

$$\eta_2 = 0{\cdot}029 \log \left(\frac{i + i_0}{i_0} \right) \qquad (1)$$

where under conditions of vigorous stirring $i_0 \sim 3$ m.amp. cm.$^{-2}$. The observation[1–3] that η_2 attains an almost constant value for i greater than 0·040 amp. cm.$^{-2}$, when hydrogen is evolved as bubbles, has been associated[1] with either critical supersaturation of the diffusion layer with dissolved molecules or saturation of chemisorption sites. Experiments reported here appear to eliminate the latter possibility.

The general effect of increasing the amount of rhodium in the Pd–Rh–H system (ref. 6 and unpub-

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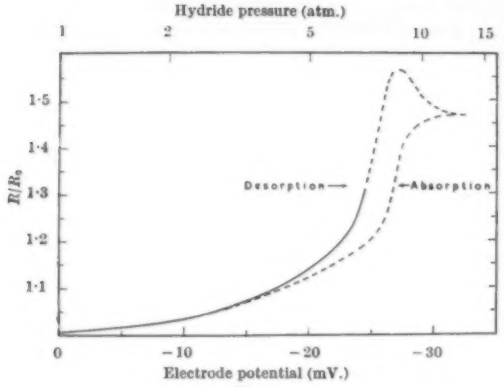

Fig. 1

lished results) resembles that of platinum in the Pd–Pt–H system[3] in increasing the hydride vapour pressure over regions where α- and β-phase hydrides co-exist (and the solids can show marked changes in hydrogen content) and is paralleled (unpublished results) by changes in electrical resistance (recorded as R/R_0 where R_0 is the resistance of the hydrogen-free alloy). Relevant relationships (25° C.) for the 23 per cent rhodium (by weight) system are shown in Fig. 1, where the desorption data were estimated with specimens of slightly less than homogeneously diffusion-controlled activity (see later). Cathodes were in the form of wire (0·0122 cm. diameter; about 10 cm. long) and the apparatus has been described[7]. R/R_0 was recorded, in conjunction with electrode potential both during and subsequent to cathodization, using an a.c. bridge[8] and measurements carried out in 0·002 N and 0·01 N hydrochloric acid to avoid complications due to solution-conduction of the bridge current[7,8]. Pre-activation was either (i) by pre-flaming at close to white heat, or (ii) by plating palladium black from aqueous palladium chloride, or (iii) by plating 'bright' palladium from aqueous tetramminepalladous nitrate under conditions recommended by Philpott[9]. All electrode potentials were zero in hydrogen-saturated solutions prior to cathodization.

As anticipated, values of $\eta_{\frac{1}{2}}$ (from steady-state values of R/R_0) attained by palladized specimens were in accord with equation 1 for $i \leqslant 0{\cdot}020$ amp. cm.$^{-2}$ ($\eta_{\frac{1}{2}} \sim 26$ mV.). Furthermore, in support of Knorr's contention[5] that the overvoltage at all platinum group cathodes (homogeneously activated) should be generally governed by the rate of diffusion of hydrogen molecules from their surfaces, pre-flamed specimens could be easily prepared which obeyed equation 1 in this range of i; not only attaining virtually identical steady-state values of R/R_0 to palladized specimens for the same i but also exhibiting virtually identical rates of decrease of $|E|$ and R/R_0 on interruption of cathodization. When $i \sim 0{\cdot}050$ amp. cm.$^{-2}$, dense clouds of tiny bubbles began to evolve from all cathodes with surfaces sufficiently active for $\eta_{\frac{1}{2}}$ to be given by equation 1 at lower current densities: with palladized specimens, $\eta_{\frac{1}{2}}$ could thereafter be increased little further, since R/R_0 never exceeded 1·2 even on increasing i to 0·260 amp. cm.$^{-2}$ (equation 1 giving $\eta_{\frac{1}{2}} \sim 56$ mV.); in one specimen R/R_0 was constant at 1·13 from 0·050 to 0·260 amp. cm.$^{-2}$. These results were not the consequence of insufficient chemisorption sites, however, because $R/R_0 \sim 1{\cdot}4$ was rapidly attained both by less surface-active pre-flamed specimens (even for $i < 0{\cdot}020$ amp. cm.$^{-2}$) and also by cathodes which had been quite thickly coated with bright palladium but which exhibited, after interruption of electrolysis, only slightly slower rates of decrease of $|E|$ and R/R_0 (over comparable ranges of values) than cathodes for which R/R_0 was not found to exceed 1·2.

It is difficult to measure differences, resulting from hysteresis over the region of co-existence of α- and β-phase hydride (unpublished results) between the absorption/desorption relationships (Fig. 1), so that the precise value of $\eta_{\frac{1}{2}}$ during cathodization must be assessed with caution. So far, however, results suggest that the limiting hydride vapour pressure, and thus the pressure of dissolved hydrogen molecules in the diffusion layer, can be as low as 10 atm., which is slightly less than that indicated from work with pure palladium[1,3]. In further experiments with this and other palladium alloys, it is hoped to define these limiting pressures even more precisely.

We are indebted to the International Nickel Co. (Mond), Ltd., for providing the palladium/rhodium alloy.

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Kinetics of Dissociation of Ethyl Halides in Self-quenching Geiger–Müller Counters

By using the experimental procedure described in ref. 1 mass-spectrometric work on electron-impact dissociation of ethyl halides C_2H_5X(X = Cl, Br, I) in a working Geiger–Müller counter has been carried out.

Simple analysis of the respective decay diagrams shown in Fig. 1 leads to the conclusion that, as in the case of saturated hydrocarbons, a regular, essentially first-order, slow decomposition process follows an initial very fast consumption of parent molecules which practically comes to an end after the

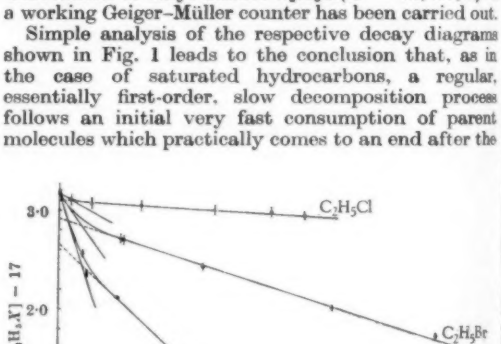

Fig. 1. Decay curves of ethyl halides used as quenching vapours

first 10^7–10^8 pulses have been registered by the counter. It is interesting to note that from our mass-spectrometric data evidence was obtained that in the case of ethyl iodide the large initial loss of quenching parent molecules amounts to about 98 per cent. For both ethyl chloride and bromide the respective values lie between 85 and 50 per cent.

Assuming that the observed fast consumption process is due primarily to strong adsorption phenomena occurring on freshly degassed electrode and glass surfaces, it seems reasonable to expect that only very few undecomposed parent molecules will reach the wall of the counter. The average life-time before decomposition for an excited polyatomic molecule is less than 10^{-12} sec. (ref. 2). The time required by the same molecule to reach the critical distance from the wall (where the probability of neutralization equals unity) being about 10^{-12} sec., it may be assumed that only about one in 10^9 molecules of ethyl halide can reach the cathode surface before decomposing. Furthermore, it is apparent that heavy organic molecules such as ethyl halides have a large probability of pre-dissociating at all energies.

In order to illustrate the primary decomposition of excited ethyl halide molecules or ions in a working Geiger counter use may be made of the following scheme:

$$(M^0, M^+)^* \underset{k'_i}{\overset{k_i}{\rightleftharpoons}} (R^0_i, R^+_i) + (R^+_j, R^0_j)$$
$$\qquad\qquad \downarrow k_{R_i} \qquad\qquad \downarrow k_{R_j}$$

where M^0 and M^+ are parent molecules or their ions; R_i and R_j—free radicals, the usual primary decomposition products; k_i and k'_i—the decomposition and recombination-rate constants; k_{Ri} and k_{Rj} rate-constants of subsequent free-radical secondary reactions.

If the concentration of the molecular species M^0 and M^+ is denoted by $[M]$ and those of $[R^0_i + R^+_j]$ and $[R^0_j + R^+_j]$ by $[R_i]$ and $[R_j]$ respectively, then:

$$\frac{\mathrm{d}[M]}{\mathrm{d}n} = k_i[M] - k'_i[R_i][R_j]$$

Now, at the beginning of the rapid-loss portion of the decay curve it may be assumed that $[M] \gg [R_i][R_j]$ which leads to a first-order equation for the rate of disappearance of parent molecules from a fresh quenching gas:

$$\frac{\mathrm{d}[M]}{[M]} = k_i \mathrm{d}n$$

where n is the total number of counts registered by the scaling unit. The straight lines, tangent to the decay curves at their origins, shown in Fig. 1, represent the kinetics of the initial decomposition of the respective quenching agents. It need scarcely be said, however, that with continued use of the counter, the composition of its quenching gas is steadily changing. The main features involved are the recombination of free radicals to form a wide assortment of heavier molecules and the adsorption-desorption equilibrium. Hence the over-all decay rate-constant is gradually approaching the k'_s value of the first-order slow-decomposition process.

The calculated rate-constants for both the initial rapid loss and the subsequent slow decomposition of C_2H_5X molecules are shown in Table 1.

Table 1. Quenching Characteristics of Ethyl Halide Vapours

Quenching molecules	C_2H_5Cl	C_2H_5Br	C_2H_5I
Filling pressure (at 298°) in mm. mercury	60	60	60
Counter volume (ml.)	71·2	71·2	71·2
Cathode	graphite	graphite	copper
Average No. of electrons/pulse, $\times 10^{-8}$	2·75 ± 0·27	7·2 ± 0·72	17·2 ± 1·7
Initial rapid decomposition rate-constant $k_p{}^r \times 10^8$/pulse	5·06	13·2	29·2
Initial rapid decomposition rate-constant $k_e{}^r \times 10^{11}$/electron	1·84 ± 0·18	1·83 ± 0·18	1·70 ± 0·17
Initial specific electron yield $S_e \times 10^{-3}$ molecules/electron	2·7 ± 0·3	2·7 × 0·3	2·55 ± 0·26
Slow decomposition rate-constant $k_p{}^s \times 10^8$/pulse	0·55	2·76	8·74

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Space Group and Unit Cell of the *m*-Bromobenzoate of Photoisopyrocalciferol

The unit cell and space group of the *m*-bromobenzoate of photoisopyrocalciferol have been determined, and an X-ray diffraction investigation with three-dimensional data is in progress. Dauben and Fonken[1] have postulated a structure for photoisopyrocalciferol based on chemical and spectroscopical investigations. The *m*-bromobenzoate has been prepared with a chemical analysis of C 72·23 per cent, H 8·17 per cent, and Br 13·58 per cent compared with the calculated values of C 72·52 per cent, H 8·17 per cent, and Br 13·79 per cent for the $C_{31}H_{47}O_2Br$ molecule. The crystals melt over the range 93·5–95° C. The crystals are colourless, flat needles with the long dimension parallel to the *b* axis. The *a* axis is perpendicular to the flat sides of the needles. Weissenberg photographs of the type *hnl* and *hk*0 have been taken showing systematic absences only of the type 0*k*0 with *k* odd. The usual intensity relationships for the monoclinic system are observed. The space group $P2_1$ is the only one consistent with the above observations and compatible with the asymmetrical molecules. The following cell dimensions have been determined from films calibrated with quartz powder patterns: $a = 21{\cdot}13$ Å.; $b = 7{\cdot}39$ Å.; $c = 10{\cdot}35$ Å.; $\beta = 92°\ 14'$.

The density calculated from the above cell dimensions assuming two molecules a cell of 1·19 gm./c.c. compares favourably with the density of 1·17 gm./c.c. determined by flotation methods.

A grant from the Research Corporation for equipment has made this preliminary work possible and is gratefully acknowledged.

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Alkali Metal Titanium Oxide Bronzes

The crystallographic relationships between $K_2Ti_2O_5$, $Na_2Ti_3O_7$ and $Na_2Ti_6O_{13}$, the structures of which have been determined by us[1], led to the belief that oxide bronzes with host lattices of formula TiO_2 might be induced to form under reducing conditions.

The reduction in hydrogen of coarsely crystalline $Na_2Ti_3O_7$ for two days at 950° C., followed by extraction with hot sulphuric and hydrofluoric acids, led

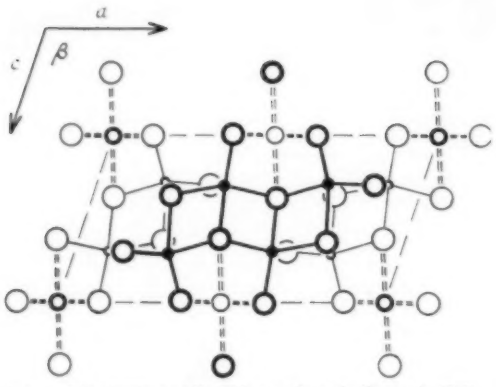

Fig. 1. The structure of Na_xTiO_2 seen in projection on to (010). The smaller circles are titanium, the intermediate ones are sodium, and the largest oxygen. The heavier outlined atoms are at the level $y = 0$, the lighter at $y = \frac{1}{2}$. The sodium–oxygen bonds are drawn as broken lines, and the unit cell is shown in outline

to the isolation of opaque, blue-black electrically conducting crystals with the empirical formula Na_xTiO_2 (x approximately equal to 0·2). The symmetry is monoclinic, and the unit cell dimensions are $a = 12{\cdot}146$, $b = 3{\cdot}862$, $c = 6{\cdot}451$ Å., $\beta = 106{\cdot}85°$, space group $C2/m$. The structure, deduced by trial and error and refined by Fourier methods as well as by a least squares treatment, is shown as a projection in Fig. 1. The titanium atoms each have a distorted octahedral grouping with oxygen, and the octahedra themselves share edges to form a double sheet which, in Fig. 1, is parallel to the a axis. These sheets are joined together by corners and this results in a new structure of formula TiO_2. The Ti—O bonds vary between 1·7 and 2·2 Å. with an average of 1·97 Å., and the oxygen–oxygen distances range from 2·4 to 3·2 Å., average 2·76 Å.

This host lattice contains within it interstitial positions which are occupied, in part only—and at random—by sodium ions each of which has four bonds to oxygen of 2·5 Å. and four longer ones 3·0 Å. Ideally speaking these positions would be cubic, but the distortions introduced by the irregular Ti—O octahedra force the sodium to adopt a virtually rectangular planar configuration.

In a discussion of the crystal chemistry of the vanadium oxides, the possibility of structural homologues with the general formula $Me_{2n}O_{5n-2}$ was pointed out[3], V_6O_{13} ($n = 3$) and V_2O_5 being the only members known at that time. It is now interesting to note that the host lattice of this sodium titanium bronze, while having a different metal ion, nevertheless has the structure of the end member of the series, $n = 2$.

Blue fibrous needles of K_xTiO_2 (x approximately 0·13) were formed by the reduction of $K_2Ti_2O_5$ under identical conditions. The tetragonal unit cell dimensions were $a = 10{\cdot}172$, $c = 2{\cdot}846$ Å., the indices showing pseudo body centring. None of the needles proved to be a single crystal, and a further detailed investigation by us is not contemplated. The structure of this substance is undoubtedly related to that of the mineral hollandite which, however, is truly body-centred tetragonal or monoclinic[3]. This structural type, previously considered to be capable of occurring as chemical compounds with bronze-like characteristics[4], is one containing continuous linear interstices, or tunnels, which enclose variable numbers of ions such as barium, potassium, or lead, all of which are considerably larger than sodium. The host cation may be one or other of the quadrivalent elements manganese, as in hollandite itself, or titanium[5]. The minor symmetry anomaly which alone distinguishes it from these published data possibly could involve order and disorder of the potassium ions in the tunnels.

One other titanium bronze, La_xTiO_3 ($0{\cdot}7 < x < 1{\cdot}0$), has been recorded, and this is isomorphous with the cubic tungsten bronzes with the perovskite structure[6]. Thus it bears little relationship to either of the two briefly described here.

We are indebted to Mr. E. S. Pilkington for the chemical analyses.

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BIOCHEMISTRY

Formation of 2,3-Dihydroxybenzoic Acid in Fermentation Liquors during the Submerged Culture Production of Lysergic Acid α-Hydroxyethylamide by *Claviceps paspali* Stevens and Hall

A strain of *Claviceps paspali* Stevens and Hall has previously been shown to be capable of producing lysergic acid α-hydroxyethylamide in submerged fermentation[1,2]. Examination of chromatograms (solvent n-butanol/acetic acid/water, 4 : 1 : 5) of culture fluids at the end of the fermentation period revealed the presence of a mobile (R_F 0·83) spot showing bright blue fluorescence, not giving the Ehrlich–Van Urk reaction for lysergic acid derivatives but giving a blue-violet colour with ferric chloride.

The substance responsible for this spot could be extracted from the culture medium acidified with concentrated sulphuric acid with two volumes of ether and was obtained crystalline after transfer to water at pH 4, re-extraction with ether at pH 1, washing the ether extract repeatedly with water and evaporating to dryness. It was recrystallized from water. The yields were of the order of 200–350 mgm. from 1 litre of culture medium, showing that the substance is a metabolite of major importance in the lysergic acid fermentation by *Claviceps paspali* Stevens and Hall. It gave a strong blue-violet colour with ferric chloride which turned red on alkalinization with sodium hydroxide. It analysed for $C_7H_6O_3$ (C, 54·65; H, 3·92 per cent; calc. C, 54·52; H, 4·01 per cent) and was identified as 2,3-dihydroxybenzoic acid by comparison with an authentic sample by chromatographic behaviour in three solvent systems

as well as by its ultra-violet and infra-red spectrum, its melting point (200° C.), mixed melting point (no depression) and its first dissociation constant (found $1{\cdot}1 \times 10^{-3}$).

2,3-Dihydroxybenzoic acid is also formed from l-tryptophan by washed mycelium of *Claviceps paspali* Stevens and Hall in replacement cultures and clearly is one of its breakdown products in this micro-organism. To our knowledge, 2,3-dihydroxybenzoic acid has not been identified as a product of tryptophan degradation in any other tryptophan derivative synthesizing micro-organism. It has, however, been shown to be formed from salicylic acid in *Aspergillus niger*[3], and in the rat[4], and to occur in Nature in the buds of *Populus balsamifera*[5] and as a glucoside in the leaves of *Vinca minor*[6].

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Identification of an Amylobarbitone Metabolite in Human Urine

Amylobarbitone (5-ethyl-5-(3-methylbutyl) barbituric acid) fed to dogs in doses of 45 mgm. per kgm. has been shown to produce 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid as the main metabolite, about 67 per cent of the original dose being excreted in this form[1].

We have recently identified this metabolite in the urine of a woman who had died 30 hr. after ingesting an unknown quantity of 'Tuinal' (equal parts of amylobarbitone and quinalbarbitone).

The metabolite was obtained by saturating the urine with ammonium sulphate, acidifying with hydrochloric acid, and continuously extracting with ether for 8 hr. A 0·5 *N* ammonium hydroxide extract of the ether was acidified with hydrochloric acid, saturated with ammonium sulphate, and re-extracted with a large volume of ether. The ether was evaporated, and the residue dissolved in a small volume of alcohol, which was spotted on Whatman No. 1 chromatography paper. The paper was developed in *n*-butanol/0·880 ammonia (3 : 1) for 4 hr.

After exposing the paper to ammonia vapour, four absorbing spots were detected with R_F's 0·65, 0·18, 0·08 and 0 respectively using ultra-violet light (2537 Å.). A permanent record was obtained by contact printing the ammonia-treated paper chromatogram using Ilford 50 reflex paper and the same ultra-violet source[2] (Fig. 1).

Fig. 1. Chromatogram developed in butanol–ammonia. *A*, 5-Ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid; *B*, total urine extract; *C*, amylobarbitone; *D*, eluted R_F 0·65 metabolite. Ultra-violet transmission photograph after exposing chromatogram to ammonia vapour

Table 1. *d*-Values of 5-ethyl-5-(3-hydroxy-3-methylbutyl) Barbituric Acid

d-Value	Intensity	*d*-Value	Intensity
9·82	20	4·225	10
8·865	80	3·525	40
6·32	20	3·425	10
6·1	20	3·27	20
5·71	70	2·925	20
5·45	100	2·795	30
5·37	50	2·71	20
4·99	10	2·67	10
4·925	30	2·56	20
4·79	10	2·525	20
4·55	100	2·175	20
4·275	20		

The highest of the spots had the same R_F as a synthetic sample of 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid run adjacently. None of the spots corresponded with amylobarbitone. The substances with R_F's 0·65 and 0·18 gave purple colours after spraying with cobalt chloride (1 per cent in acetone) and exposing to ammonia vapour[3] and were the only ones showing greatly increased absorption after ammonia vapour treatment, indicating an intact pyrimidine ring.

The R_F 0·65 urine metabolite was eluted with alcohol acidified with hydrochloric acid, and the X-ray

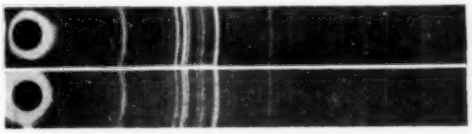

Fig. 2. X-ray diffraction patterns of 5-ethyl-5-(3-hydroxy-3-methylbutyl) barbituric acid. 2*a*, Synthetic; 2*b*, urine metabolite R_F 0·65

diffraction pattern compared with that of the synthetic material, using copper $K\alpha$ radiation. Patterns were identical (Fig. 2*a* and *b*). The *d*-values calculated from this pattern are given in Table 1.

The nature and origin of the other absorbing spots are being investigated and will be reported later.

It is of interest that no unchanged amylobarbitone or quinalbarbitone was detected in this urine.

We are indebted to Eli Lilly and Co., Ltd., for a gift of the synthetic 3-hydroxy amylobarbitone.

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Chromatography and Nucleotide Sequences of Deoxyribonucleic Acid synthesized in *Salmonella typhimurium* after Irradiation with Ultra-Violet

It has been shown[1,2] that ultra-violet irradiation produces in some kinds of bacteria, for example, *Escherichia coli* B/r and *Salmonella typhimurium*, a temporary inhibition of deoxyribonucleic acid (DNA) synthesis. The synthesis is resumed after 30–60 min. and proceeds at a rate similar to the control, but the surviving bacteria are growing only in mass, having lost their ability to form colonies. One of the possible explanations for such behaviour is that DNA synthesized after temporary inhibition is different from the normal one. That this might be so is supported by Kanazir *et al.*[3], who found that whereas DNA from non-irradiated *Sal. typhimurium* could reduce the damage to an irradiated culture, that from an irradiated culture could not, and by Hudnik and Glisin[4], who showed that the thermal denaturation pattern followed by viscosity and ultra-violet absorption measurement was different in the two DNA's.

Ord and Stocken[5] using the usual analytical procedures were unable to distinguish between DNA of thymus from normal and irradiated animals, but a difference could be established by chromatography on 'Ecteola' columns according to the method of Bendich *et al.*[6]. The same method was therefore used for characterization of DNA from normal and irradiated *Sal. typhimurium*.

The growth of bacteria and the irradiation conditions were the same as described in ref. 3. At zero time one-half of the bacteria were irradiated with an LD_{50} dose of ultra-violet light delivered from Hanovia mercury vapour lamp and phosphorus-32 (1 μc./ml. culture) added 120 min. later. At this time there is reason to suppose that the bacteria are synthesizing DNA at the same rate as their controls. Phosphorus-32 was added to the control bacteria 90 min. from zero time and both irradiated and control samples were gathered 90 min. after the addition of phosphorus-32. They were then washed and DNA isolated according to the method of Zamenhof[7] using 15 per cent 'Dupanol' for the lysis of the cells. Ribonucleic acid (RNA) was removed by digestion with ribonuclease and ethoxyethanol precipitation as described by Kirby[8]. The possible contamination with RNA was checked by measuring radioactivity in the supernatant after digestion of the DNA with alkali. No radioactivity could be detected in this fraction. The protein content was less than 0·5 per cent when tested according to Lowry[9].

Chromatography on 'Ecteola' was performed as described by Ord and Stocken[5]. Two mixing chambers in series were used for gradient elution and the elution pattern followed by measurement of optical density at 260 mμ. The control and irradiated samples of DNA were eluted in parallel from the common supply of solvent. The fractions were pooled and analysed for radioactivity in a liquid Geiger counter and for DNA by the method of Ceriotti[10].

Nucleotide sequences were estimated according to the method of Burton and Petersen[11] on unlabelled DNA samples prepared in the same way as described here.

If the amount of ultra-violet absorbing material eluted in the main fractions in the control and irradiated DNA are compared (Table 1) it can be seen that more is eluted in the irradiated DNA in fractions I*A*, *B* and III and less in fractions II and IV, as compared with the control. Although small, the differences are consistent in both experiments.

Table 1. Chromatography on 'Ecteola' of DNA from Normal and Ultra-Violet Irradiated *Salmonella typhimurium*

Fraction	E_{260} as percentage of control	
	Exp. 1	Exp. 2
1 *A*	119	127
1 *B*	104	105
2	90	79
3	103	104
4	86	89

Fig. 1 represents the specific activities of pooled sub-fractions plotted against the composition of the eluant. It can be seen from the elution pattern that the radioactive fractions of the irradiated DNA start with the elution at a lower concentration of sodium chloride, and that the specific activities of the fractions eluted with sodium chloride gradient are higher in the irradiated DNA. It is difficult to see why this is so since both control and irradiated bacteria synthesize practically the same amount of DNA in the same time. It is possible that although the net synthesis is roughly the same in the two cases, the DNA formed in the irradiated bacteria is less stable[12] and the turnover correspondingly greater. There are smaller differences in the subsequent fractions, and at the end of ammonia

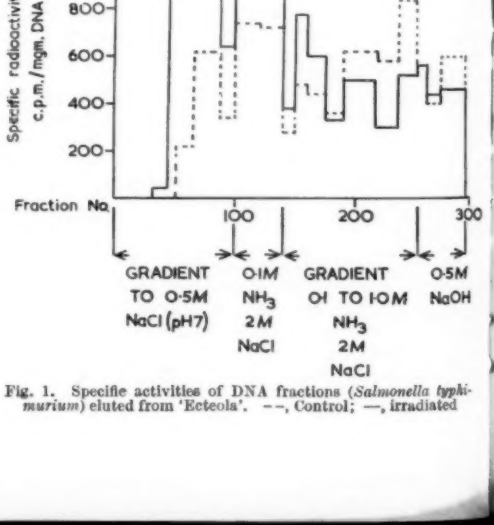

Fig. 1. Specific activities of DNA fractions (*Salmonella typhimurium*) eluted from 'Ecteola'. ––, Control; —, irradiated

gradient and in the sodium hydroxide sub-fractions the specific activity of the irradiated DNA is lower than that of the control.

The results of the estimation of frequencies of some nucleotide sequences are summarized in Table 2. No difference was found in the frequency of the following sequences: C, T, T_2p_2, T_4p_3, T_5p_4, CT_4p_5, T_3Cp_3 and C_2Tp_2. But some of the nucleotides containing more cytosine appear to be more frequent in the irradiated DNA while some nucleotides with more thymine seem to be more frequent in the control. It will be noted that the TpC/CpT ratio is changed from 1·33 to 1·16 and this adds further support for the idea that irradiation has modified the structure of the DNA (cf. Burton[13]). Relevant to this are the observations of Beukers and Berends[14], who found a dimerization of thymine which had been exposed to ultraviolet irradiation *in vitro*, and of Wacker *et al.*[15], who noted a change in 2^{14}C-thymine of bacterial DNA irradiated *in vivo*.

Table 2. NUCLEOTIDE SEQUENCES OF DNA FROM NORMAL AND IRRADIATED *Salmonella typhimurium*

Product*	Pyrimidine (moles/100 gm. atoms of phosphorus) Control DNA	Irradiated DNA
T_2P	2·62 ± 0·06	2·4 ± 0·1
TpC	3·10 ± 0·09	2·98 ± 0·06
T_3Cp_2	2·32 ± 0·10	2·12 ± 0·07
T_4Cp_4	0·75 ± 0·02	0·52 ± 0·07
C_2p	3·54 ± 0·06	3·65 ± 0·07
C_2p_2	0·77 ± 0·04	0·94 ± 0·07
CpT	2·33 ± 0·02	2·57 ± 0·06
$C_2T_2p_2$	0·97 ± 0·04	1·17 ± 0·08
T	5·2 ± 0·06	5·2 ± 0·1
C	5·80 ± 0·02	5·8 ± 0·1
T_2p_2	0·90 ± 0·1	0·87 ± 0·07
T_4p_3	0·41 ± 0·04	0·39 ± 0·03
T_5p_4	0·20 ± 0·03	0·19 ± 0·02
T_3Cp_3	1·11 ± 0·07	1·11 ± 0·11
T_4Cp_4	0·40 ± 0·08	0·35 ± 0·02
C_2Tp_2	2·41 ± 0·06	2·48 ± 0·14

* Nomenclature according to Burton and Petersen (ref. 11).

We are grateful to Dr. J. Lascelles and Dr. R. Tucker for help with the irradiation and growth of the organism and to Dr. K. Burton and Dr. M. Lunt for advice on the determination of nucleotide sequences. We wish to acknowledge financial support from the Department of Scientific and Industrial Research and the International Atomic Agency, and to one of us (T. H.-P.), from the Institute of Nuclear Sciences, Belgrade.

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Determination of Argininosuccinic Acid by Ion-Exchange Chromatography

RECENTLY, an unknown amino-acid excreted in the urine by two mentally defective children[1] was identified as argininosuccinic acid (ASA)[2]. This is unstable in weakly acid aqueous solutions and cyclizes[3]. More recently Westall[4] has shown that under these conditions two separate types of anhydride (B and C) are formed, rapidly at high but more slowly at room temperatures.

For the original metabolic studies of ASA in the mentally defective children[5], the gradient elution fraction-collecting method of Moore and Stein[6] employing the cation exchange resin 'Dowex 50' of 4 per cent cross-linking was used. Under these conditions ASA emerged cleanly from the column as two separate peaks which we now know corresponded to the two anhydride forms B and C. The rate of chromatographic development of these columns is slow (6–8 ml./hr.) and ASA is subjected to a pH of between 3 and 4 for a period of about 60–70 hr. at 30–50° C. This time is presumably long enough for the ASA to be completely converted to the two anhydride forms which emerged from the columns in the position on the flow sheet between the peaks of leucine and tyrosine.

More recently we have attempted to measure ASA using an automatic self-recording amino-acid analyser[7] which has been built in this Department. This method used the 'Amberlite' cation-exchange resin *CG*–120 of 8 per cent cross-linking and the columns are operated at a flow-rate of 30 ml./hr. ASA is not eluted from the 150 cm. column with the pH 3·25 buffer at 30°, but after changing to the pH 4·25 buffer at 55° a small amount of free ASA emerges after the passage of the new buffer front and this is followed by a slowly rising baseline and the emergence of several partially separated peaks immediately after the phenylalanine position. Pure samples of either of the B and C anhydrides when treated similarly are stable and give each time a single peak at 5 and 45 ml., respectively, after the centre of the phenylalanine peak. There is no sign of any reconversion to ASA and the baseline is unaltered. We believe, therefore, that after only 10 hr. at 30° with the pH 3·25 buffer the original ASA is not fully converted to the anhydride forms; conditions are then changed to pH 4·25 at 55°, but before a new equilibrium is attained a mixture of ASA and its anhydrides is eluted from the column.

On the 50 cm. column used for the estimation of basic amino-acids in biological fluids, operated at pH 4·19 and 30°, free ASA emerges at 25 ml. and the anhydrides B and C at 50 and 60 ml. respectively. About 5 per cent of anhydride was found in the analysis of a sample of ASA on the 50 cm. column, but this amount could well have been present in the original sample itself. It is therefore possible to estimate ASA in pure solution on the 50 cm. column, but this is of little practical use since the presence of the acidic and neutral amino-acids, which are usually present in biological fluids, grossly interfere at the beginning of the run between 10 and 40 ml.

In order to overcome these difficulties we have made use of the fact that ASA in solution can be readily converted to the anhydride forms by standing for 48 hr. at pH 2 and the anhydrides are stable providing the pH does not rise above 5. Provided that the cyclization is complete then the formed anhydrides can be determined on the 50 cm. column where they emerge conveniently between the large

peak of combined acidic and neutral amino-acids and the peak given by phenylalanine and tyrosine at about 80 ml. In a test of the experimental conditions by analysis carried out on the 50 cm. column, 1 μmole of a sample of ASA gave, after standing at *p*H 2 for 24 hr. at 20°, 0·04 μmole of ASA, 0·04 μmole of *B* and 0·69 μmole of *C*. After increasing the time of standing to 48 hr. the values were 0·01 μmole of ASA, 0·04 μmole of *B* and 0·71 μmole of *C*.

Provisionally we are applying a correction factor to compensate for the 75 per cent recovery of ASA as the anhydrides.

In practice, samples of urine and deproteinized plasma are brought to *p*H 2 by the dropwise addition of 2 *N* hydrochloric acid and are allowed to stand at room temperature for 48 hr. prior to analysis.

Miles Laboratories Inc. and the Ames Co., Ltd., provided a generous grant for the construction of the automatic amino-acid analyser which was built in the Medical Unit by Mr. C. E. Glover and Mr. G. Marshall.

One of us (D. C. C.) is indebted to the Drummond Trust for a senior fellowship.

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Chromatographical Separation of Carbohydrate *O*-Isopropylidene Derivatives

During investigations on fluorocarbohydrates, it became necessary to examine the purity of small amounts of *O*-isopropylidene derivatives. Hitherto, for such compounds in the carbohydrate series, there has been no reliable chromatographical method of separation from fully protected or nearly related hydroxylated compounds. We have found, however, that separations can be achieved rapidly and easily by reversed-phase, ascending chromatography on cellulose acetate strips (20 × 4·5 cm., as used for electrophoresis and obtainable from Oxo, Ltd., Queen Street Place, London, E.C.4).

Strips were washed thoroughly in methanol and dried in air. Compounds (50–800 μgm. in ethanol) were applied as spots to the washed strip which were then developed by ascending chromatography with a mixture of methanol and water (6/4 v/v). The separation was achieved in 2 hr. at 2–5° C.

After drying for 20–25 min. in air at room temperature, compounds can be detected in two ways:

(*a*) Developed chromatograms are immersed in a solution[1] of 2,4-dinitrophenylhydrazine (0·4 per cent w/v) in 2 *N* hydrochloric acid. Carbonyl, *O*-isopropylidene and *O*-benzylidene compounds appear as deep yellow zones which are further intensified when the chromatogram is washed briefly in aqueous sodium hydroxide (10 per cent w/v).

(*b*) Alternatively, the developed chromatograms are floated on the surface of a solution[2] (1 per cent w/v) of potassium permanganate in 1 *N* sulphuric acid for 2 min. and immersed for a further minute. The chromatogram is then washed in running water, when the components appear as sharply defined purple-brown spots, which turn brown on standing. This is sensitive to about 150 μgm. of such compounds as 1,2 : 5,6-di-*O*-isopropylidene-D-glucose.

Table 1

Substance	R_F	No. of free hydroxyl groups
1,2 : 4,5-di-*O*-isopropylidene-3-mesyl-D-fructose	0·15	0
1,2-*O*-benzylideneglycerol	0·23	1
Methyl *O*-isopropylidene-DL-glycerate	0·28	0
1,2 : 3,4-di-*O*-isopropylidene-6-tosyl-D-galactose	0·32	0
Methyl 2,3-*O*-isopropylidene-α*β*-D-ribofuranoside	0·48	1
1,2 : 5,6-di-*O*-isopropylidene-D-glucose	0·52	1
1,2 : 3,4-di-*O*-isopropylidene-D-galactose	0·55	1
1,2-*O*-isopropylideneglycerol	0·65	1
Methyl DL-glycerate	0·66	2
(±)-butan-1,3-diol	0·73	2
1,2-*O*-isopropylidene-D-glucofuranose	0·80	3
2,3,4,6-tetra-*O*-methyl-D-glucose	0·90	1
Methyl 2,3,6-tri-*O*-methyl-α*β*-D-glucoside	0·98	2
3-*O*-methyl-D-glucose	0·98	4

As seen from the list of R_F values in Table 1, the method in general can be used to distinguish fully protected sugars from those with one or more free hydroxyl groups. In general, unsubstituted sugars move at the solvent front.

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Isolation of Histones(?) from *Staphylococcus aureus*

Any theories that try to give an explanation of the function of histones have had in the past to take account of the reported absence of histones from bacteria[1,2]. If histones are solely concerned with cell differentiation or 'gene suppression' in multicellular organisms, then one might expect them to be absent from bacteria and protozoa[3,4]. But if, on the other hand, they have an even more fundamental function, related to the function of deoxyribonucleic acid (DNA) in all cells, then they should also be present in unicellular organisms.

In the present investigation an attempt was made to extract basic proteins from bacteria, following a modification of the procedure routinely used in our laboratory for the isolation of histones from cell nuclei[5].

The bacteria, *Staphylococcus aureus*, were washed with 4 per cent acetic acid, and then defatted and dried by washing several times with ethanol and then with ether. This procedure denatures many of the non-basic proteins, rendering them insoluble on extraction with acid. The acid-soluble proteins were extracted by grinding the dried bacteria with glass powder in the presence of 0·1 *N* sulphuric acid, and then centrifuging. Large amounts of non-protein material extracted were removed by dialysis against 0·1 *N* sulphuric acid, and the proteins were precipitated from the dialysed extract by the addition of seven volumes of ethanol, and dried with ethanol followed by ether.

Some of the extracted material was subjected to starch-gel electrophoresis in the presence of 4 *M* urea

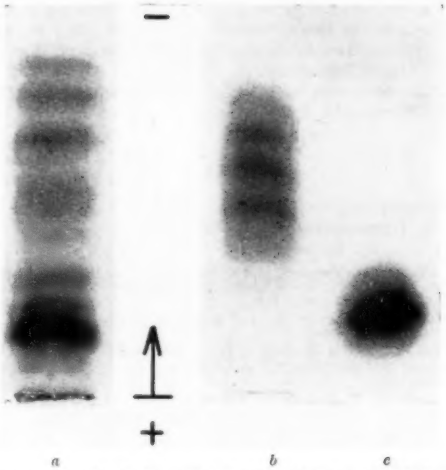

Fig. 1. Starch-gel electrophoresis of proteins extracted from *Staphylococcus aureus* with 0·1 *N* sulphuric acid : (*a*) total protein, (*b*) basic protein fraction, (*c*) non-basic fraction

using a modification of the technique developed by Neelin and Neelin for use with histones[6]. The gel, rolled 1 mm. thick on a glass plate and coated afterwards with paraffin wax, was prepared with 0·014 *M* acetate buffer containing the urea, giving a *p*H within the gel of 6. After migration, the gel was stained with amido black 10*B* and showed the presence of at least eight components (Fig. 1). The direction of migration of the slower components is reversed if the *p*H of the gel is increased above 6·5.

Those components which were strongly basic, like histones, with isoelectric points well above *p*H 7 were separated from the others by using carboxymethyl-cellulose. The protein mixture, dissolved in bicarbonate buffer *p*H 7, was applied to a very short carboxymethyl-cellulose column previously washed with buffer. Under these conditions the non-basic components run through the column while the more basic components are retained. The column was washed with water, and then the basic proteins eluted with about 1 ml. of 0·1 *N* hydrochloric acid.

Starch-gel electrophoresis of these two fractions, recovered from the eluates by ethanol precipitation, shows the complete separation of the faster components from the remainder, which, although extracted by acid, do not have isoelectric points above *p*H 7 (Fig. 1).

Amino-acid analysis of both fractions, by acid hydrolysis followed by paper chromatography (two successive runs in one direction using an *n*-butanol, acetic acid, water system and detection of the spots by ninhydrin reagent), indicated that the faster, more basic fraction was very similar to unfractionated ox-thymus histone[7]. It contained particularly large amounts of lysine, arginine, alanine, leucine and proline with relatively small quantities of acidic amino-acids, histidine and tyrosine, while the slower, less basic fraction was quite different. It is concluded from the above results, from ultra-violet absorption and from solubility characteristics that the proteins in the faster electrophoretic fraction are very similar indeed to histones.

Thus it is clear that these bacteria contain basic proteins like the histones found in the higher forms of life. Comparable results have been obtained with *Micrococcus lysodeikticus*. These basic proteins may also occur in the DNA-protein fraction previously isolated by Vendrely *et al.* from *Escherichia coli*[8].

It is not yet possible to say whether all the basic protein components demonstrated here have come from association with the DNA or the ribonucleic acid of the bacteria. But somewhat similar components have recently been shown to occur in ribosomes prepared from *Escherichia coli*[9].

We wish to thank Dr. J. F. Wilkinson for providing us with liberal supplies of bacteria.

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Detection and Distribution of Enzymes for oxidizing Thiocysteamine

The intermediate role of the persulphide analogues (*R*—SSH) of cysteine and cysteamine in the biological conversion of cystine and cystamine into the corresponding sulphinates and thiosulphonates has been suggested in ref. 1. This view has been emphasized by the detection of an enzyme, or enzyme system, able to convert thiocysteamine (the persulphide analogue of cysteamine : NH_2—CH_2—CH_2—SSH) into thiotaurine (*R*—SO_2SH) and hypotaurine (*R*—SO_2H) in the presence of air[2]. Careful study of this reaction merits attention since it may throw light on the first steps of the metabolic pathway of cystine and cystamine.

In our earlier work[2] thiotaurine and hypotaurine were detected in the incubation mixtures by using labelled substrate and by submitting the final deproteinizate to chromato-radioautography or chromato-scanning. Although providing good results, these methods are time-consuming, expensive, and cannot be used as routine procedures for the isolation of the enzyme. Thus we have sought simpler methods for obtaining activity values. Since thiotaurine is the main product of the enzymic oxidation of thiocysteamine, methods have been tried for determining this compound. The cyanolysis method[3] and ion-exchange chromatography[4,5] seemed simple enough and were adapted to the determination of thiotaurine in our incubates. The results obtained with these two methods have been compared with those obtained by scanning radiochromatograms.

The enzyme was extracted from pig kidney by fractionation between 20 and 60 per cent ammonium sulphate[2]. Thiocysteamine was produced in the incubates by adding cysteamine hydrochloride and elementary sulphur[2]. The experimental conditions are reported in Tables 1 and 2. The distribution of the enzyme has been investigated by comparing the amount of thiotaurine produced under standard con-

Table 1. Time-course of the Enzymic Conversion of Thiocysteamine into Thiotaurine, as determined by Three Different Methods

Enzyme: 32 mgm. of proteins of 20–60 per cent ammonium sulphate saturation fraction of pig kidney homogenate dissolved in 2 ml. water. Thiocysteamine: 50 μmoles containing 8 μc. of sulphur-35. Phosphate buffer *p*H 7·4: 0·6 ml., 0·1 *M*. Final volume, 3 ml.; Temperature, 38°. Values reported in μmoles of thiotaurine produced.

Time of incubation (hr.)	Cyanolysis	Ion-exchange chromatography	Chromato-radio scanning
2	2·3	1·9	2·3
4	2·5	2·1	2·8
6	2·9	3·3	3·0

Table 2. Distribution of Thiocysteamine Oxidizing Enzyme

Values in μmoles of thiotaurine produced after 2 hr. under standard conditions by using the cyanolysis procedure. Conditions were the same as described in Table 1, with the exception that the enzyme was substituted with 2 ml. of a homogenate of 1 part of the organ with 5 parts of phosphate buffer, and that thiocysteamine was not labelled.

	Kidney	Liver	Muscle	Heart
Rat	1·35	1·72	0·91	0·85
Rabbit	1·23	0·50	0·23	0·25
Pig	1·76	2·56	—	0·48
Cattle	2·07	0·90	0·50	0·88
Horse	4·88	1·21	0·71	0·90

ditions by the same amount of homogenate of various organs. Details are reported in ref. 2.

The cyanolysis technique was that described by Sörbo[3]. Various controls have been performed and it was found that interfering substances were removed by the addition of 'Dowex 50'. The cyanolysis determination was performed as follows: The final incubation mixture (3 ml.) was deproteinized with 0·3 ml. 100 per cent trichloracetic acid and added to 0·3 gm. of 'Dowex 50 ×4', 200–400 mesh, in the H^+ form. After shaking and centrifuging, an aliquot of the supernatant was added with 2 ml. 1 *N* ammonia and 1·5 ml. of 0·1 *M* potassium cyanide. After 30 min. in a boiling water-bath, the solution was cooled, added to 1 ml. ferric nitrate reagent[3] and read at 470 mμ.

Ion-exchange determination of thiotaurine was performed on the trichloracetic filtrate, without the addition of the resin, following the procedure of Stein[5]. The eluant was 0·1 *M* citric acid in 0·2 *M* sodium chloride at 37°. By using this solution, thiotaurine is eluted together with taurine, far from other disturbing amino-acids[4]. Since our enzyme preparation was free from taurine, the peak which developed with ninhydrin in the area of thiotaurine was calculated as pure thiotaurine.

The procedure for scanning uni-dimensional radiochromatograms has been described in ref. 2. We have observed that part of the radioactivity is recovered in the trichloracetic precipitate at the end of the incubation. This is probably due to exchange of thiocysteamine with thiols and disulphides of proteins[6]. For that reason values for thiotaurine have been calculated by comparing the radioactivity of the area occupied by thiotaurine in the chromatograms with the radioactivity of a known amount of the substrate. In our earlier papers radioactivity of thiotaurine was compared with the radioactivity distributed in the whole chromatogram. The yield of thiotaurine, by using the present procedure, is consequently lower than that reported in ref. 2 and is more accurate.

As shown in Table 1, the procedure for cyanolysis gives results which are comparable with those obtained by ion-exchange and by chromato-radio-scanning. This finding permits the use of the very simple procedure of cyanolysis for the detection of the enzyme. Table 2 shows the first results of the study of the distribution of the enzyme in various organs and animals. These preliminary results suggest a wide distribution of the enzyme and a general importance of the reaction. Moreover, they indicate that the horse kidney is the best source for the extraction of the enzyme.

This work has been aided by a grant of the Consiglio Nazionale delle Ricerche.

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Presence of Haptoglobin in Synovial Fluid

Electrophoretically classified α- and β-glycoproteins have been shown to be normal constituents of synovial fluid[1]. Evidence based on immunoelectrophoresis and Cohn fractionation of human traumatic effusions also suggests the presence of most of the serum proteins in synovial fluid[2,3]. Individual biologically active serum proteins have also been found, namely, some enzymes[4], siderophilin[3], ceruloplasmin[3], and the thyroxine-binding protein[5]. The accepted blood-coagulating factors, however, were found to be absent from normal bovine fluid[6].

In an experiment using starch-gel electrophoresis, the patterns obtained with bovine serum and synovial fluid were found to be identical. Electrophoretic zones obtained for both were albumin, α-globulin, 4β-globulins, a slow α-globulin and γ-globulin[7]. Since the hæmoglobin-binding protein, haptoglobin, migrates electrophoretically as an α-globulin, it seemed likely that this glycoprotein might be a normal constituent of bovine synovial fluid. Its presence in the normal fluid was further suggested by its relatively low molecular weight (85,000).

Since haptoglobin complexes hæmoglobin[8], its presence is readily ascertained by determining the electrophoretic migration of the haptoglobin–hæmoglobin complex. Thus, at a *p*H of 7·0–7·5 the complex migrates anodically while free hæmoglobin is immobile. The amount of haptoglobin may then be expressed in terms of the hæmoglobin-binding capacity.

In the present work the electrophoretic procedure was that described in detail by Zak *et al.*[9] using a dilute agar medium. A 0·25 per cent solution of agar (certified pure) in 0·05μ veronal–citrate buffer of *p*H 7·3 (10·3 gm. sodium barbital and 2·1 gm. citric acid per litre) was transferred to an 8·3 cm. × 10·2 cm. glass basin containing a sheet of parchment paper (No. 72 vegetable parchment powder paper, Upjohn Co.). After electrophoresis for 45–50 min. at 250 V. and 40–60 m.amp., the agar plate was dried under an infra-red lamp. The dried parchment paper was stained with a 1 : 1 mixture of benzidine (saturated solution in glacial acetic acid) and hydrogen peroxide (3 per cent solution). The hæmoglobin-containing bands gave a typical blue reaction.

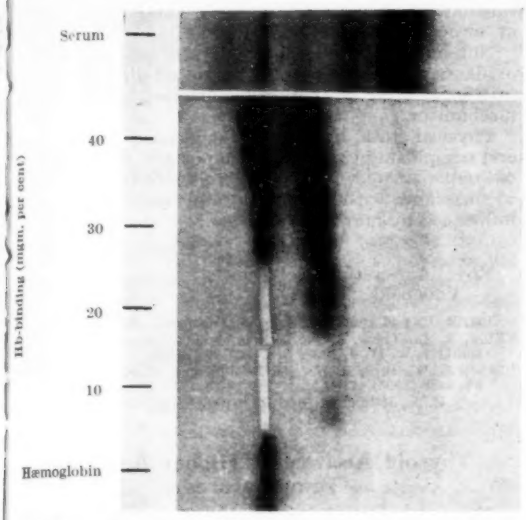

Fig. 1. Electrophoretic determination of hæmoglobin-binding capacity in human serum

Graded series of bovine sera and concentrated synovial fluids were prepared so as to contain 5, 10, 20, 30 and 40 mgm. per cent of hæmoglobin. For these a standard solution (5 gm. per cent) was previously diluted to contain 50, 100, 200, 300 and 400 mgm. per cent. Then 0·01 ml. aliquots of the diluted standard solutions were mixed with 0·1 ml. portions of serum or concentrated (1 : 10) synovial fluid. Bovine synovial fluids (5 ml.) were first incubated with hyaluronidase prior to concentration by lyophilization. Small strips (0·5 mm. × 8 mm.) of Whatman No. 3 *MM* filter paper were soaked in the samples and then were placed on the agar gel approximately 1·5 in. from the cathodic end.

In a preliminary experiment, human serum was used (Fig. 1) to establish the applicability of this electrophoretic technique to the determination of haptoglobin. Fig. 2 demonstrates the presence of haptoglobin in bovine synovial fluid. Free hæmoglobin remained at the origin while the haptoglobin-bound hæmoglobin migrated between the α- and β-fractions. The average hæmoglobin-binding capacity of seven samples of concentrated synovial fluid was approximately 10 mgm. per cent. When based on the total protein content of synovial fluid (average 1 gm. per cent) the binding-capacity was 1 mgm. of hæmoglobin per gm. of total protein. Bovine serum gave a range of 10–20 mgm. per cent. However, it was impossible to collect bovine serum from the slaughter-house without some degree of hæmolysis. Determinations of the content of hæmoglobin showed the presence of 5–10 mgm. per cent in the serum. Therefore, the actual hæmoglobin-binding capacity for serum averaged 20 mgm. per cent for 7 animals or 2·5 mgm. of hæmoglobin per gm. of total protein.

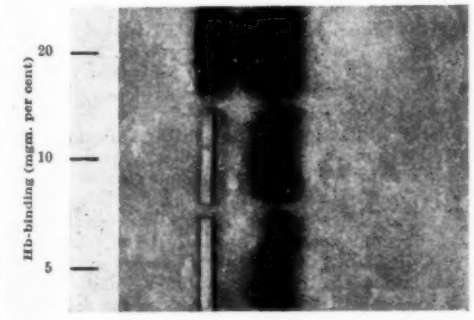

Fig. 2. Electrophoretic demonstration of haptoglobin in normal bovine synovial fluid

On the basis of the preceding results, it is apparent that the glycoprotein, haptoglobin, is a normal constituent of bovine synovial fluid. The hæmoglobin-binding capacity of synovial fluid, expressed in mgm. hæmoglobin/gm. total protein, is approximately 40 per cent of the binding capacity of the serum.

This work was supported by grants from the U.S. Public Health Service (*A*-532) and the Michigan Chapter, Arthritis and Rheumatism Foundation.

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PHYSIOLOGY

Hormonal Control of Oxidized Flavour in Milk

In two recent papers the importance of natural copper in producing oxidized flavour in milk has been stressed[1,2]. The pronounced effect of adding copper salts to induce oxidized flavour, as well as the inhibiting effect of a great many compounds with copper-binding abilities, has been recognized for a long time. Contamination with copper occurs easily in metal equipment with which the milk has been in intimate contact[3]. Contribution from equipment was considered to be the main origin of copper in milk, and thus the importance of natural copper for development of oxidized flavour may have been overlooked. High copper values are found in carefully sampled milk[1,4], and particularly high values in milk from the early lactation period[1]. Elevated content of natural copper in this period seems to indicate a hormonal regulation of copper metabolism. The copper-catalysed oxidation in milk may be enhanced or depressed, and factors of this kind will combine to give the final result. The addition of copper sulphate to milk is a method used to evaluate these factors, or the so-called susceptibility, and is of practical importance. The relative effect of natural copper contents is lessened. Indications of hormonal influence on oxidized flavour in milk are reported here.

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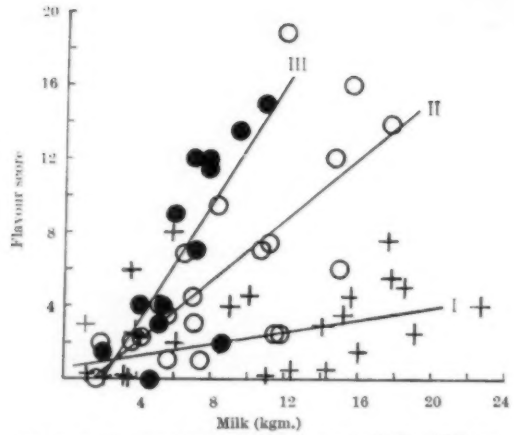

Fig. 1. Flavour score versus milk yield. Groups I, II and III are cows with 4 or more, 2 and 3, and 1 gestations, respectively. Coefficients of correlation are 0·34, 0·70 and 0·74. Scores of group I are significantly lower than those of groups II and III

The results are obtained by scoring milk from the College farm herd, sampled from each cow during milking. The samples were left overnight in the cold store to be judged next day by a single taster. Oxidized flavour scores were magnified by adding copper sulphate to the milk samples. Thus, sufficient grading was attained, and this made statistical treatment possible. Good correlations were obtained for flavour score versus milk yield on the same day, especially when the cows of the herd were divided into groups due to the number of gestations they had had. The interrelations given in Fig. 1 for a scoring in September are retained for all parts of the year and lactation period. Very recent freshened cows may, however, disturb the regression, particularly cows in their first lactation period. The regression coefficient in the latter case has a negative value in the first part of the lactation period.

The liability of a certain farm to produce milk with oxidized flavour must then, besides frequency of recent calvings, also depend on age-distribution of the animals. The correlation coefficient between the sum of monthly flavour scorings of untreated milk last year and the average age of animals in 23 herds in the district was found to be − 0·52, with regression coefficient significantly deviating from zero. Milk sampled from farms was drawn at point of delivery.

When a group of young cows was followed through a 5-wk. period, 8 cows showed heat. The average score throughout the 3-wk. sex cycle could then be calculated and plotted. The curve showed its summit at, or near, the day of heat (Fig. 2).

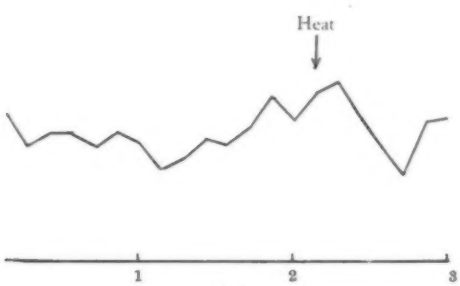

Fig. 2. Flavour score during the three-week sex cycle

Not only natural copper but also pro- and anti-oxidants may contribute to the noted differentiation in Fig. 1 so far as they are controlled by the same mechanism.

Physical state, like size of fat globules, organizing and complexing compounds (important for excretion of copper in milk), or tocopherols, are factors likely to interfere with oxidation and may be factors influenced by hormonal activity.

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Thyroid Activity at Higher Ambient Temperatures

Thyroid activity is increased in animals exposed to cold with release of thyroid hormone[1] and both the output from, and the uptake of, iodine-131 by the thyroid is augmented[2,3], but the blood-level of protein-bound iodine[4] and the epinephrine-potentiating effect of thyroxine[5] are decreased. Work on the evolution of thermoregulation reveals that birds and baby mammals suffer from defective thermoregulation[6,7] and there is increased activity of the adrenergic system on exposure to high ambient temperature[8,9]. In the present investigations, thyroidal activity of birds and baby rabbits has been investigated after exposing them to high ambient temperature.

Baby rabbits (weighing about 300 gm.) and pigeons (weighing about 275 gm.), maintained at an average temperature of 32° C., served as controls, and they were exposed to 45° C. and 48° C. respectively in constant-temperature chambers for 2·5 hr., the standard error of temperature variation being within ± 0·03° C. Protein-bound iodine (PBI) level of serum was estimated[10] and a histological study of the thyroid was carried out. After exposure to heat, the PBI-level increased in blood in both groups. Histological work on the thyroid showed an increase in acinar cell height (140 per cent) with an overall release of the acinar colloid in pigeons exposed to heat, while in the exposed baby rabbits there is no significant increase in acinar cell height, although there is a slight release of the colloid (Table 1).

On exposure to cold there is an excessive release of thyroid hormone, but the serum PBI-level falls due to greater utilization of the hormone in the cold[11,12]. In the present work, the high concentration of serum PBI, associated with histological evidence of release of thyroid hormone, might indicate a decrease in peripheral utilization of the latter, although this point has not yet been verified experimentally. Thyroid acinar cell height in pigeons exposed to heat is 140 per cent of the control, while in the baby rabbits there is no difference, indicating increased thyrotrophic hormone secretion by the anterior pituitary in pigeons, probably as a result of the stimulation of hypothalamus. In the baby rabbits a small depletion of thyroid colloid with unaffected acinar cell height indicates little release of thyrotrophic hormone. This difference in behaviour might be due to the fact

Table 1. Normal Pigeons and Baby Rabbits exposed to Warm Environment

Animals, with number in parentheses		Ambient temperature	Serum PBI in μgm. per 100 ml. ± standard deviation	Increase (per cent)	Thyroid acinar cell height in μ ± standard deviation	Increase (per cent)	Colloid
Pigeon	(15)	32° C.	4·37 ± 1·02	143	3·31 ± 0·42	140	Overall release
Pigeon	(15)	48° C.	6·23 ± 0·90		4·62 ± 0·63		
Rabbit	(12)	32° C.	6·32 ± 1·1	24	4·40 ± 0·60	101*	Release, but comparatively less
Rabbit	(12)	45° C.	7·84 ± 1·0		4·46 ± 0·51		

* Not statistically significant.

that these baby rabbits have passed the stenothermic phase of thermoregulation and the small release of thyroid hormone is probably a direct temperature effect unlike that in pigeons[8]. The cause of the increased serum PBI concentration and its physiological effect on tissue respiration are being investigated.

We thank M. S. Das for his interest and advice.

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Effect of Quinones on the Uptake of Oxygen by Red Cells

It is well recognized that many oxidizing agents will convert hæmoglobin to methæmoglobin. Among these are the quinones[1]. Vitamin K_3, however, could have an additional effect through its role in electron transport[2], although it is difficult to envisage a complete electron chain since mature red cells possess no cytochromes.

In this work human erythrocytes were separated from plasma and the other formed blood elements and incubated in a Warburg manometric apparatus in the usual way[3]. Vitamin K_3 (0·2 mgm./flask), 1 : 4-naphthoquinone (0·2 mgm./flask), which has a similar structure but no known physiological role, and dicoumarol (0·3 mgm./flask), an analogue of vitamin K_3 and also a potent inhibitor of vitamin K_3 reductase[3], were added from the side-arm at the beginning of the experiment as indicated.

Red cells have a predominantly glycolytic metabolism. The small amount of oxygen uptake (Fig. 1) is probably accounted for by the auto-oxidation of hæmoglobin to methæmoglobin. Normally the latter so formed is reduced by reduced diphosphopyridine nucleotide (DPNH) derived from the oxidation of 3-phosphoglyceraldehyde, the reaction being catalysed by methæmoglobin reductase. As a result of this reduction mechanism only 1–2 per cent of the total hæmoglobin is normally present as methæmoglobin. Following the addition of vitamin K_3 or 1 : 4-naphthoquinone there was a rapid oxidation of hæmoglobin to methæmoglobin within 2 min. and a corresponding oxygen uptake. Thereafter the rate of oxygen utilization fell but was still greater than in the absence of the quinones. After 3 hr. incubation 45 per cent of the hæmoglobin was present as methæmoglobin compared with 1·7 per cent in the absence of quinones. Dicoumarol has no effect on oxygen uptake nor does it lead to the formation of methæmoglobin.

In the absence of glucose the rate of uptake of oxygen fell after 1 hr. and the level of methæmoglobin in the presence of vitamin K_3 or 1 : 4-naphthoquinone was 58 per cent after 3 hr. This may be attributed to the gradual depletion of endogenous glucose, which makes available a supply of DPNH and TPNH for methæmoglobin reduction. Similar results were obtained using lysed red cells. Under these conditions uptake of oxygen is much reduced, possibly due to configurational changes in the proteins or co-factor dilution.

Elsewhere[3] we have shown that compounds which lead to the production of methæmoglobin in the cell stimulate both uptake of oxygen and the amount of glucose oxidized via the pentose pathway with the production of TPNH, which then reduces methæmoglobin. There is at present some controversy as to whether DPNH and TPNH are linked to the same or different enzymes.

Since vitamin K_3 and 1 : 4-naphthoquinone had identical effects on oxygen uptake and led to the formation of excess methæmoglobin in the cells while dicoumarol did not depress oxygen uptake, it would

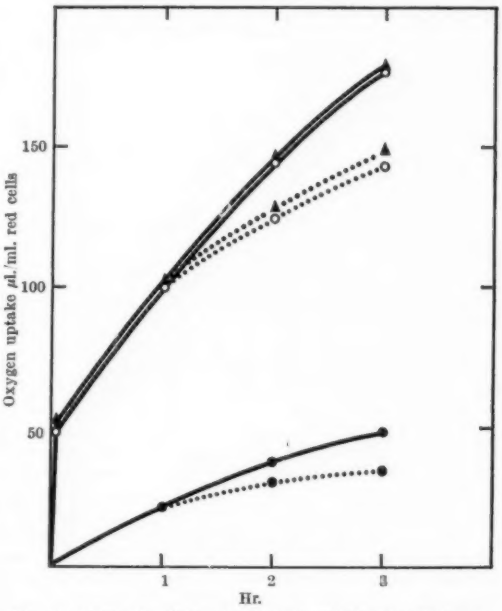

Fig. 1. Effect of quinones in presence and absence of glucose on oxygen uptake of 1 ml. of packed red cells in isotonic buffer *p*H 7·4 at 37°
●—●, red cells + glucose only; ● · · · ●, red cells without glucose added; ○—○, red cells + 1 : 4-naphthoquinone + glucose; ○ · · · ○, red cells + 1 : 4-naphthoquinone without glucose added; ▲—▲, red cells + vitamin K_3 + glucose; ▲ · · · ▲, red cells + vitamin K_3 without glucose added

seem that the effect of vitamin K_3 in stimulating oxygen uptake by the red cells is a result of the oxidation of hæmoglobin.

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Plasma Albumin Catabolism in Experimental Nephrosis

Glomerular filtration and tubular re-absorption of plasma albumin occur as part of the normal function of the mammalian kidney[1]. The fate of the re-absorbed protein is less certain. Oliver *et al.*[2] have presented evidence that enzyme systems capable of catabolizing certain of the plasma proteins exist in the renal tubule cell, suggesting that re-absorbed protein is broken down in transit through the cell. However, we have demonstrated that in the normal rat[3] and dog[4], bilateral nephrectomy depresses the breakdown of plasma albumin to only a limited extent. The amount of plasma albumin contained in the renal tubules of rats with experimental nephrosis is much greater than normal, and Goodman and Baxter[5] maintain that this is due to enhanced glomerular filtration and subsequent tubular re-absorption of greater than normal quantities of plasma protein. We have reasoned that if the kidney has any important role in plasma albumin catabolism it should be demonstrable under these conditions of exaggerated albumin reabsorption.

Rats were made nephrotic by 5–10 daily subcutaneous injections of the aminonucleoside of puromycin[6]. Such rats had plasma albumin concentrations of 8–15 mgm./ml., and they excreted 100–250 mgm. of albumin daily in their urine. With 8–10 injections most rats had lipæmic sera, œdema, and ascites. With 5–7 injections the serum was often clear, there was less œdema, and only occasional ascites. Animals were used within 15 days of the start of injections. Rat plasma albumin was iodinated with iodine monochloride labelled with iodine-131 (ref. 7) and 'screened', according to McFarlane[8], for 2–4 days before use. All rats received 0·3–0·8 μc. of screened iodoalbumin intravenously immediately after nephrectomy or sham nephrectomy. Urine was collected quantitatively when available. After 8 or 24 hr. rats were killed and ground in a meat grinder, and the non-protein radioactivity extracted from the ground rat tissue with trichloroacetic acid. The methods used have been described previously[3].

The results are summarized in Table 1. Normal rats broke down 24 per cent of the injected iodoalbumin in 24 hr. Following nephrectomy the rate of breakdown in the non-nephrotic rats averaged 20 per cent. This is a significant change, and carries a P value of 0·01–0·005. In previous experiments with screened iodo-albumin[3] we found depression in breakdown following nephrectomy of 0–15 per cent; and experiments with perfused kidney[3] indicated that up to 15 per cent of the albumin turnover in the body may occur in kidneys.

Table 1. Effect of Bilateral Nephrectomy on Iodoalbumin Breakdown in Normal and Nephrotic Rats

Duration of exp. (hr.)	Condition of animal	Bilateral nephrectomy	No. of rats	Iodoalbumin breakdown Injected radioactivity (per cent) Range	Average
24	Nephrotic	−	22	16–29	23
		+	21	4–15	11
24	Normal	−	16	20–28	24
		+	12	16–26	20
8	Nephrotic	−	13	8–15	12·5
		+	13	2·5–9·0	4·6

Nephrotic rats broke down 23 per cent of the injected iodoalbumin in 24 hr. and about 14 per cent in 8 hr. On removal of the kidneys breakdown was markedly reduced, averaging 11 and 5 per cent in 24 and 8 hr. respectively. In nephrotic rats the turnover of plasma albumin is very rapid (estimated half-life 4–12 hr.). This is due primarily to the urinary excretion of albumin, which in a day amounts to several times that of the circulating plasma albumin. 30–80 per cent of the injected radioactive iodoalbumin was excreted as such in the urine within 24 hr.

It should be stressed that the breakdown of radioactive iodoalbumin recorded in Table 1 cannot be directly equated with the rates of breakdown of the total exchangeable plasma albumin. In rats without proteinuria the breakdown of iodoalbumin reflects primarily the catabolism of plasma albumin, and during the period of these experiments iodoalbumin breakdown is approximately proportional to the rate of albumin catabolism. On the other hand, in animals with extensive proteinuria, labelled iodoalbumin is removed very rapidly from the circulation and the specific activity of circulating albumin drops to within 10–20 per cent of the initial value within 24 hr. In the experiments described here, the nephrotic rats retained from less than 20 to 50 per cent of the injected radioactivity in the exchangeable albumin pool. On the other hand, in animals without proteinuria (including nephrectomized nephrotics) 70–80 per cent of the injected radioactivity was retained. Thus the effect of removal of kidney on the rate of plasma albumin breakdown in the nephrotic rat cannot be readily estimated in quantitative terms from our results. To evaluate quantitatively the effect of nephrectomy on the rate of albumin breakdown the turnover kinetics of albumin in animals with and without kidneys must be evaluated. For this purpose the size of the vascular and extravascular albumin pool, the rates of albumin excretion, breakdown, and synthesis, as well as the rates of exchange of albumin between the vascular and extravascular pools must be determined. Most of these parameters are being measured in work now in progress in our laboratory.

In spite of the limitations indicated above, our results indicate that albumin catabolism is greatly reduced following nephrectomy in nephrotic rats. In these animals the initial fractional rate of iodoalbumin degradation, and thus of plasma albumin breakdown, is as high as in normal rats. On nephrectomy the initial fractional breakdown of iodoalbumin is less in the nephrotic rat than in the normal (with or without kidneys) and much less than in the nephrotic with kidneys. This indicates that the rate of albumin catabolism in nephrotic rats without kidneys is much less than in all the other groups.

It stands to reason that if the only change following nephrectomy were the elimination of albumin loss in

the urine, the fractional rate of iodoalbumin breakdown should increase. This would be so, since the radioactivity of the circulating albumin pools remains high, and thus the specific activity of the breakdown products would be, on the contrary, higher than in rats the iodoalbumin of which is rapidly depleted by proteinuria. Our results show the fraction broken down is greatly decreased.

The best explanation of our findings is provided by the assumption that in the nephrotic rats, kidneys are a major site of plasma albumin breakdown. Thus, the results strongly suggest that in amino-nucleoside nephrotic rats with extensive proteinuria, removal of kidneys markedly decreases plasma albumin breakdown, and that a major, and possibly predominant, part of plasma albumin breakdown in this type of acutely nephrotic animal occurs in the kidney.

This work was supported by grants from the National Heart Institute, U.S. Public Health Service, and the American Heart Association.

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Inhibitory Properties of the Catecholamines

For some years considerable attention has been focused on the inhibitory action of γ-aminobutyric acid (GABA). One of the first systems used to demonstrate this property involved inhibition of the spontaneous discharge of the slowly adapting stretch receptor neurone of the crayfish[1,2]. This bio-assay procedure has been used to investigate the inhibitory characteristics of many other compounds[2-4], but none reported to date has proved more active than GABA. A number of aliphatic amino- and guanidino-acids have, however, shown activity approaching that of GABA, and pre-requisites for activity appear to be the presence in the molecule of an acidic and a suitably separated basic function. Such general characteristics are to be found among the catecholamines. The inhibitory characteristics of these substances were therefore investigated.

Pacifastacus leniusculus (Dana) was the crayfish used and the method was essentially that described by Florey[1] and by Elliott and Florey[5]. The commercially obtained compounds were made up in 'crayfish saline'[6] at *p*H 6·5–7 and the 'threshold blocking concentration' was determined; this was the concentration which would cause a transient (*c.* 20 sec.) block of the spontaneous discharge. The activity of any compound was expressed in arbitrary units, defined as the reciprocal of the concentration, in m.moles/litre, which produces a threshold block when applied to the stretch receptor neurone.

Results comparing the inhibitory activity of GABA and the catecholamines are given in Table 1. 3-Hydroxytyramine showed a much higher order of activity than GABA or the other catecholamines on every crayfish preparation used. Although there was great variability in the response of the different preparations to the substances tested, it was repeatedly shown that a given substance tested under two code numbers on a given preparation gave concordant results.

Table 1. Inhibitory Activity of GABA and Some Catecholamines on the Crayfish Stretch Receptor Neurone

Substance	No. of preparations	Activity in units defined in text (mean)	(range)
GABA	18	80	15–206
3-hydroxytyramine hydrogen chloride	15	6,250	566–41,580
L-noradrenaline bitartrate, water	7	110	5–468
L-adrenaline bitartrate	5	65	< 4·5–185

Our results on L-noradrenaline bitartrate and L-adrenaline bitartrate, indicating an activity comparable with that of GABA, are in conflict with those of Elliott and Florey[5], who reported that these compounds were inactive at a concentration of 1 mgm./ml. (less than 0·3 unit in each case). These workers used crayfish of the species *Orconectes virilis*, and did not specify the number of trials. The results we have obtained with many aliphatic amino- and guanidino-acids agree remarkably well with those of Edwards and Kuffler[3], who used both *O. virilis* and *Procambarus alleni*; it therefore seems unlikely that the difference in species of crayfish used can account for the difference in results between ourselves and Elliott and Florey.

The ability of various agents to block the inhibitory effects of 3-hydroxytyramine and of GABA was significantly different. Picrotoxin (10^{-3} *M*) blocked about 60 per cent of the activity of GABA, but was only slightly effective against 3-hydroxytyramine. Chlorpromazine ($1{\cdot}5 \times 10^{-4}$ *M*) and dibenzylene (8×10^{-4} *M*), on the other hand, were ineffective against GABA but blocked 3-hydroxytyramine almost completely. These results suggest that more than one receptor site on the stretch receptor neurone is involved.

The significance of these results remains to be assessed by work on other systems. The high activity of 3-hydroxytyramine lends support to the many investigators who have suggested that it may be an effector substance in its own right as well as a precursor of adrenaline and noradrenaline.

The work was supported by Federal Mental Health grants No. 609–5–128 and No. 609–5–130 to E. G. and P. L. McGeer; and by a National Research Council of Canada grant to H. McLennan.

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Acquired Tolerance to Homografts produced by Homologous Spleen Cell Injection in Adult Mice

The dosage of skin homografts has been shown to be a factor in their survival. While small homografts show a prolonged survival-time when compared with medium-sized grafts, the application of large or near total body size homografts results in increased survival time[1,2]. Billingham and Sparrow also showed that intravenous injection of living homologous dissociated epidermal cells caused a two- to three-fold increase in the survival of skin homografts which were afterwards placed on rabbit hosts[3]. Because of our success in producing acquired tolerance to outcross skin in adult mice using parent-F_1 hybrid parabiosis[4,5], the following experiment was designed to study the effects of dosage of homologous antigen on the future recipients of skin homografts in adults, reasoning along lines that the success of parabiosis was primarily a dosage phenomenon.

Groups of adult *C3H* female mice were injected intravenously with a spleen cell suspension obtained from either $(Ce \times C3H)F_1$ hybrid, $(A \times C3H)F_1$ hybrid. *A*, or $(C_{57}(BL) \times C3H)F_1$ hybrid mice. The contents of three donor spleens were suspended in 1 ml. of Ringer's solution such that each ml. contained 300 to 340 million nucleated cells. Injections were made intravenously through a 30-gauge needle placed in the tail vein. One week later a 2·5 × 3·5-cm. full thickness, homologous, abdominal skin-graft taken from donors corresponding to the strain of injected spleen cells was placed on the back of host animals. Each graft was rotated 180° to facilitate identification of viability and checked daily for gross signs of rejection.

Table 1 demonstrates the differences in graft behaviour between the experimental groups. $(C3H \times Ce)F_1$ hybrid skin, accepted by *C3H* hosts at 30–48 days of age, is not accepted at 120–130 days of age unless receiving spleen cells when 30–48 days old. In the case of *C3H* mice injected with $(A \times C3H)F_1$ spleen and grafted with $(A \times C3H)F_1$ skin, the final behaviour of the skin homograft depended on the dosage of spleen injected one week before grafting. Seven of ten grafts placed, following an injection of 300–340 million cells, showed no signs of destruction except in two cases where there was shrinkage to approximately 50 per cent of the original size and a less dense hair-distribution than the other 5 successful homografts (Fig. 1). These latter homografts have been viable for four and a half months. In the other 3 instances the homografts were rejected following a prolonged survival. In the strain combinations, $A \longrightarrow C3H$ and $(C_{57}(BL) \times C3H)F_1 \longrightarrow C3H$, the

Fig. 1. *C3H* female mice bearing $(A \times C3H)F_1$ female skin-graft of 4·5 months duration. Animal on the left is one of five showing complete tolerance. Animal on the right is one of two, the graft of which is 50 per cent of the original size of grafted abdominal skin

groups receiving intravenous spleen displayed prolonged survival of grafts but no permanent tolerance.

In inbred-F_1 hybrid parabiosis of adult mice a cellular exchange between partners can result in tolerance of the inbred animal to the outcross tissue of its F_1 hybrid partner. The success in provoking a tolerant state depends on the genetic disparity between inbred and F_1 hybrid[4,5]. It would seem logical, in addition, that the dosage afforded by linking the animals is an important factor in the success of this method. The results obtained in this experiment confirm those described by Billingham and Sparrow[3] on the effect of intravenous injection of cells on graft survival. Our results indicate that high doses of homologous cells alter the subsequent capacity to react against these cells, analogous to the demonstration of immunological paralysis to pneumococcal polysaccharide by Felton[6]. The dosage effect was best demonstrated when strong histocompatibility differences were minimized by the use of hybrid

Table 1

No. of animals	Host strain	Strain donor spleen*	No. of cells injected	Strain donor graft	Host age in days when grafted	Mean survival time of grafts (days)	No. tolerant
10	*C3H* ♀			$(C3H \times Ce)F_1$ ♀	37–55	24·8†	8
6	*C3H* ♀			$(C3H \times Ce)F_1$ ♀	120–130	18·2	0
14	*C3H* ♀	$(C3H \times Ce)F_1$ ♀	0·2–0·3 × 10^8	$(C3H \times Ce)F_1$ ♀	120–130		14
10	*C3H* ♀			$A \times C3H$ F_1 ♀	37–55	12·5	0
6	*C3H* ♀	$(A \times C3H)F_1$ ♀	1·5–1·7 × 10^8	$A \times C3H$ F_1 ♀	37–55	23·4	0
10	*C3H* ♀	$(A \times C3H)F_1$ ♀	3·0–3·4 × 10^8	$A \times C3H$ F_1 ♀	37–55	19·7‡	7
14	*C3H* ♀			*A* ♀	37–55	8·9	0
6	*C3H* ♀	*A* ♀	3·0–3·4 × 10^8	*A* ♀	37–55	16·1	0
6	*C3H* ♀			$(C_{57}(BL) \times C3H)F_1$ ♀	37–55	8·7	0
6	*C3H* ♀	$(C_{57}(BL) \times C3H)F_1$ ♀	3·0–3·4 × 10^8	$(C_{57}(BL) \times C3H)F_1$ ♀	37–55	13·6	0

* Host animals were between 30–48 days of age when injected with homologous spleen.

† Mean survival time of two rejected homografts.

‡ Mean survival time of three of ten rejected homografts.

animals. A spectrum of results was obtained depending on the genetic disparity between these hybrid to pure line grafts. $(C3H \times Ce)F_1$ skin on *C3H* took with minimal doses of spleen in adults. The strains *Ce* and *C3H* have the same *H*–2 locus. A large dose of spleen was required to allow the acceptance of homografts from strains differing unilaterally at the *H*–2 locus with $(A \times C3H)F_1$ and *C3H*. In the combinations $(C_{57}(BL) \times C3H)F_1 \longrightarrow C3H$ and $A \longrightarrow C3H$, a unilateral and bilateral *H*–2 locus difference respectively, even this large dose of spleen cells was insufficient to produce tolerance.

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Effect of Diethylstilbœstrol on the Serum γ-Globulin in the Guinea Pig

IT has been reported previously that the active œstrogens, both natural and synthetic, are strong stimulants of the phagocytic activity of the reticulo-endothelial system[1–3]. It has also been reported that cortisone depresses reticulo-endothelial phagocytosis, lowers the total and differential white cell blood counts, and reduces the serum γ-globulin to about half the normal level[4].

The present work was carried out to ascertain whether diethylstilbœstrol, which is a strong stimulant of the reticulo-endothelial phagocytes, raises the level of γ-globulin in the serum.

Thirty-six male guinea pigs of 450–660 gm. body-weight were used in these experiments. Sixteen of the animals were used as controls of which 9 were given no treatment and 7 received 1·5 ml. arachis oil intraperitoneally daily for 6 days. The remaining 20 animals each received 0·5 mgm. diethylstilbœstrol in 1·5 ml. arachis oil intraperitoneally once daily for 6 days. The serum γ-globulin was estimated in all the animals ; in the arachis oil controls and in those given diethylstilbœstrol this was carried out on the seventh day after treatment commenced.

The procedure was as follows : From each animal 2 ml. of blood was withdrawn by cardiac puncture, placed in a centrifuge tube and allowed to clot. The specimen was then centrifuged at 3,000 r.p.m. (r.c.f. 1,533*g*) for 20 min. and the supernatant serum removed by pipette. The serum proteins were then separated by paper electrophoresis, using a vertical tank, a barbitone buffer of *p*H 8·6, and Whatman filter paper No. 3 *MM*. Each separation was carried out on 0·01 ml. of serum using a current of 240 V. for 12 hr. The paper strips were dried, then stained with light green for 15 min. and excess stain removed with acetic acid until a clear background was obtained.

The relative distribution and the density of the serum protein bands were recorded using an 'Elphor' densitometer, and from the readings a graph was constructed on 1 mm. paper. The area enclosed by the curve representing the total serum protein was cut out and weighed ; from this the area enclosed by the γ-globulin curve was then cut out and weighed separately.

Table 1. EFFECT OF DIETHYLSTILBŒSTROL ON THE SERUM γ-GLOBULIN LEVEL IN THE GUINEA PIG

No. of animal	Body-weight (gm.)	Weight of graph	
		Area of total serum protein (gm.)	Area of γ-globulin (gm.)
Untreated controls			
1	660	0·93	0·090
2	570	0·72	0·045
3	640	0·82	0·068
4	630	0·79	0·060
5	615	0·84	0·068
6	600	0·73	0·063
7	630	0·83	0·076
8	625	0·82	0·058
9	620	0·85	0·070
Mean ± *S.D.*	621 ± 25·3	0·81 ± 0·063	0·069 ± 0·013
Arachis oil controls			
10	630	0·87	0·073
11	645	0·81	0·075
12	610	0·74	0·060
13	600	0·76	0·066
14	590	0·79	0·052
15	600	0·81	0·058
16	595	0·73	0·049
Mean ± *S.D.*	610 ± 17·3	0·79 ± 0·048	0·062 ± 0·010
Animals which received 0·5 mgm. diethylstilbœstrol daily for 6 days			
17	625	1·086	0·230
18	590	0·929	0·173
19	610	1·022	0·184
20	630	1·055	0·213
21	580	1·012	0·182
22	640	1·044	0·203
23	650	1·031	0·221
24	600	0·954	0·184
25	625	1·052	0·215
26	600	1·011	0·197
27	600	0·965	0·188
28	450	0·851	0·146
29	510	0·894	0·159
30	560	0·922	0·166
31	640	1·023	0·184
32	510	0·925	0·166
33	620	1·084	0·215
34	600	1·057	0·206
35	640	1·089	0·207
36	480	0·915	0·165
Mean ± *S.D.*	588 ± 57·2	0·996 ± 0·071	0·191 ± 0·024

The results are shown in Table 1. It will be noted that in the untreated controls the mean weight of the graph area of total serum protein is 0·81 gm. and that of the γ-globulin is 0·069 gm. The arachis oil controls show little if any variation from the results in the untreated controls. In the animals which received treatment with diethylstilbœstrol the mean weight of the graph area of total serum protein is increased to 0·996 gm. and that of the γ-globulin to 0·191 gm. These results represent an elevation of the total serum protein-level of 23 per cent above that of the control groups. This increase is composed principally of serum γ-globulin, which in fact has risen 177 per cent above normal, whereas the remaining serum proteins as a group have increased only 9 per cent. From this evidence it appears that when the reticulo-endothelial system is stimulated by diethylstilbœstrol the serum γ-globulin is increased to about 2¾ times the normal level.

Since the development of antibodies is considered to be closely related to the serum γ-globulin, it seems likely that the great increase of the latter should be accompanied by a corresponding rise in the antibody-forming potential of the animal. This has now been confirmed by further researches which are at present being prepared for publication.

XUM

In these investigations we gratefully acknowledge financial assistance from the Tobacco Manufacturers' Standing Committee and the Medical Research Council.

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HISTOLOGY

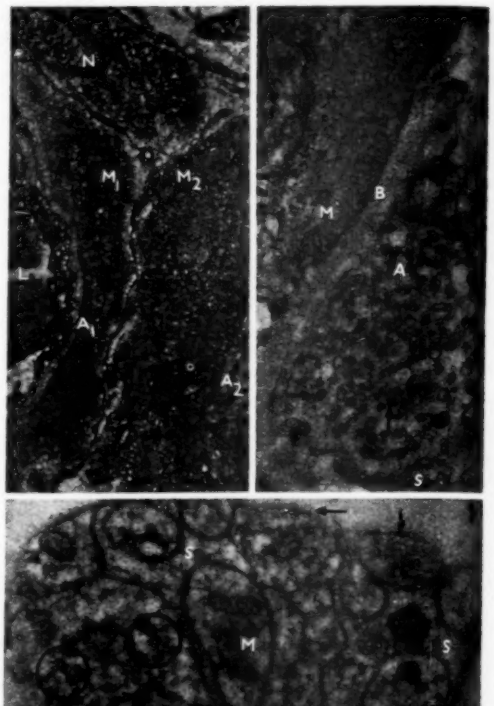
Fine Structure of the Arteriolar Nerves in the Guinea Pig Pancreas

During the course of an investigation into the effects of cobalt chloride on the endocrine pancreas of the guinea pig (Esterhuizen and Lever, in the press), our attention was arrested by certain fine structural features—which we believe have not hitherto been described—in the nerve fibres associated with the pancreatic arterioles.

According to Mitchell[1] the nerve supply to the pancreas is a mixture of post-ganglionic sympathetic fibres from the cœliac plexus and some vagal fibres. These nerves reach the gland by way of a peri-arterial plexus in company with the vessels of supply, and for the most part their subsequent distribution is alongside the branching arterial tree between the acini and ducts of the exocrine tissue and within the islets of Langerhans. It is likely that the pancreatic innervation is almost entirely vasomotor, although Lacy[2] has reported, and we also have seen, the occasional axon in direct contact with an endocrine cell.

One may commonly observe groups of unmyelinated axons lying together with a variable amount of connective tissue in the peri-vascular spaces around the small arteries (Fig. 1) in any part of the pancreas. At a fine structural level the well-known axon–Schwann cell relationship[3,4] can be seen in most of the nerve fibres present. That is to say, several axons are enwrapped by the plasma membrane of the same Schwann cell (Fig. 3), each being suspended by mesaxonal invaginations of this membrane. There is nevertheless a number of nerve fibres which are incompletely shrouded by Schwann plasma membrane, and, but for a thin surface covering of basement membrane, these axons could be described as naked over their exposed areas (Fig. 3).

Naked axons of this sort are seen frequently in close approximation to the muscular layer of the arterioles in the guinea pig pancreas. In electron micrographs the plasma membranes of opposing axon and smooth muscle cell are separated by a space which may either contain a single layer of a material having the appearance of basement membrane (Fig. 2), or be lined by this material both on its axonal and muscular sides. This space was found to range from 100 to 400 mμ in width. Although closer neuro-vascular approximation has not yet been observed, the high concentration of micro-vesicles and mitochondria[5] (Figs. 2 and 3) in many of the naked axons would suggest that in these situations nerve fibres are at, or near, their points of termination. Perhaps the synaptic arrangements of peri-vascular nerves are similar to those noted by Fawcett and Selby[6] at nerve endings in the turtle atrium in which contact between pre- and post-synaptic elements is not as intimate as at nerve terminals in many other situations.



Electron micrographs of specimens taken from guinea pig pancreas at intervals following intravenous injection of cobalt chloride (25 mgm./kgm. body-weight). Figs. 1 and 2, 24 hr.; Fig. 3, 2 hr. after injection

Fig. 1. Unmyelinated nerves (*N*) within a peri-vascular space around two small arteries A_1, A_2; lumen of first artery (*L*); smooth muscle cells M_1 and M_2 in walls of vessels. Note dense granular material in axons (*N*) (× 11,250)

Fig. 2. Naked axon (*A*) separated by a space containing 'basement membrane' (*B*) from the plasma membrane of a smooth muscle cell (*M*). Most of the micro-vesicles in the terminal axoplasm contain dense granular material (↑). Note Schwann cytoplasm (*S*) (× 40,500)

Fig. 3. Peri-arterial axons invested by Schwann cytoplasm (*S*). Note areas (↑) where axons are covered only by basement membrane. Concentrations of mitochondria (*M*) and micro-vesicles are common in these axons. In the encircled areas several micro-vesicles are seen to contain dense granular specks (× 27,000)

The micro-vesicles, found in high concentration within the terminal axoplasm of these pancreatic peri-vascular nerves, have a diameter of 350 (±50) Å. In the normal animal, although the occasional one of these vesicles was seen to contain a dense granular material, the majority of them are without visible content in electron micrographs. However, in guinea pigs examined at intervals up to five days after injection of cobalt chloride, a high proportion (at 24 hr.—the majority) of the micro-vesicles exhibit a filling of this dense material (Figs. 1 and 2). These facts might suggest: (1) that cobalt had some specific and local action on the peri-vascular nerve terminals; or (2) that filling of a large proportion of the terminal vesicles may be a local

manifestation of the response to a non-specific but stressful action of cobalt. As the neurones in question are vasomotor and presumably sympathetic post-ganglionic in nature, they can be expected to elaborate noradrenaline or a comparable substance. In tissues such as the adrenal medulla[7,8] and the carotid body[9], work has already indicated that the catecholamine transmitter agent is visualized in electron micrographs as a dense granular substance parcelled up within vesicles before release from the cell. It is therefore conceivable that the osmiophile granular material seen within some of the micro-vesicles at the termination of these arteriolar nerves in the pancreas (Fig. 2) may be visible evidence of the presence of such a transmitter substance.

One of us (J. D. L.) acknowledges a Royal Society equipment grant.

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HÆMATOLOGY

Relationships between Red Blood Cell Potassium Concentrations, Medial Corpuscular Fragility and Hæmoglobin Type in Merino and Southdown Sheep

Within any breed of sheep there are animals which have red blood cells containing a high concentration (*c.* 80 m.equiv./litre of red blood cells) or a low concentration (*c.* 20 m.equiv./litre of red blood cells) of potassium[1]. The two types are simply inherited, the latter being dominant[2].

Low-potassium Merino sheep characteristically have a very low concentration of potassium in their erythrocytes (*c.* 12 m.equiv./litre of red blood cells) and this is associated with a very low gene frequency for high potassium concentration (approx. 0·07)[3]. This breed appears to be particularly well adapted to the semi-arid regions of Australia. This could be associated with the observation that low-potassium sheep consume less water and void less urine than high-potassium animals when both types are kept under controlled conditions[4].

Although potassium concentrations in red blood cells remained remarkably constant during long periods in sheep kept under controlled conditions[5] it has been shown recently[6] that four-fold increases in the concentration of potassium in the erythrocytes of Merino sheep in the field can occur, and that this is associated with a marked lowering of the hæmatocrit value and red blood cell count. The increase in potassium concentration appears to be correlated with the severity of the anæmia and has only been recorded so far in low-potassium animals. The nature of this correlation is being investigated at present.

If a hæmolytic process associated with the swelling of the red blood cells is involved, there could be differences in the median corpuscular fragility between low- and high-potassium cells. This preliminary point has now been investigated in Merino and Southdown sheep of known hæmoglobin type[7] and red blood cell potassium type. Median corpuscular fragility determinations were carried out using the method described by Dacie[8].

Initially, the median corpuscular fragility of the red blood cells of 28 Merino ewes (23 low potassium, 5 high potassium) was examined on three occasions and a significant ($P < 0{\cdot}1$ per cent) difference between low potassium (median corpuscular fragility = 0·695) and high potassium (median corpuscular frequency = 0·625) animals was found. The between animal variation was greater than the within animal variation ($P < 0{\cdot}1$ per cent), and animals with the lowest concentrations of potassium in their red blood cells within the low potassium group appeared to have the highest median corpuscular frequency values within the group.

This initial observation was followed up with an experiment in which 21 Merino sheep were selected and divided into three groups—low potassium (7 animals with red blood cell potassium ion concentrations below 17·0 m.equiv./litre), high potassium (9 animals with more than 17·0 m.equiv./litre), high potassium (5 animals). The median corpuscular fragility was determined on each animal on 10 separate occasions. The results are given in Table 1.

A significant difference in median corpuscular fragility between low potassium and high potassium animals was again demonstrated in this second experiment ($P < 1{\cdot}0$ per cent), but although the difference between the high low potassium and low potassium group was in the direction expected from the high potassium results it was not significant. In the high potassium group one of the animals was of the type classified as $K\epsilon\beta^5$—a type not common in British breeds—and the remaining four animals were of the type classified as $K\epsilon\gamma$—the normal high potassium type in British breeds of sheep. This $K\epsilon\beta$ animal has a significantly lower median corpuscular fragility ($P < 5{\cdot}0$ per cent) than the remaining $K\epsilon\gamma$ type animals.

A similar examination of the red blood cells of 7 high potassium and 8 low potassium Southdown sheep has also been carried out and a significant difference ($P < 1{\cdot}0$ per cent) between the median corpuscular fragility of the two cell types has been found.

Although no obvious correlation between median corpuscular fragility and hæmoglobin type was observed there was a correlation between hæmoglobin

Table 1. Hæmoglobin Type, Red Blood Cell Potassium Type and Median Corpuscular Fragility in Merino Sheep

	Hæmoglobin type	No. of animals	Mean median corpuscular fragility ± S.E.	Mean median corpuscular fragility ± S.E.	Mean median corpuscular fragility ± S.E.
High potassium	*AB*	3	0·637 ± 0·019		
	B	2	0·661 ± 0·024	0·647 ± 0·015	0·647 ± 0·015
High low potassium	*A*	6	0·703 ± 0·014		
	AB	2	0·685 ± 0·024		
	B	1	0·700	0·691 ± 0·011	
Low low potassium	*A*	1	0·698		
	AB	5	0·721 ± 0·015		
	B	1	0·706	0·716 ± 0·013	0·706 ± 0·008

Levels of significance for differences in median corpuscular fragility between high potassium and low potassium, $P < 1{\cdot}0$ per cent; high potassium and high low potassium, $P < 5{\cdot}0$ per cent; high potassium and low low potassium, $P < 1{\cdot}0$ per cent; and between high low potassium and low low potassium, not significant. Effect of hæmoglobin type, not significant; variation within groups of animals less than variation between groups of animals, $P < 0{\cdot}1$ per cent.

Table 2. Hæmoglobin Type and Mean Red Blood Cell Potassium Concentrations in Merino and Southdown Sheep

Hæmoglobin type	Low potassium: Mean red blood cell potassium concentration in m.equiv./litre		High potassium: Mean red blood cell potassium concentration in m.equiv./litre	
	Merino	Southdown	Merino	Southdown
A	(10) 21·7 ± 1·52			(1) 92·3
AB	(17) 16·6 ± 1·17	(4) 18·5 ± 2·41	(2) 79·9 ± 3·41	(3) 80·1 ± 2·78
B	(13) 14·7 ± 1·34	(6) 14·1 ± 1·97	(6) 76.2 ± 1·97	(3) 72·3 ± 2·78
	* N.S.	N.S.	N.S.	† N.S. *

* $P < 1·0$ per cent; † $P < 5·0$ per cent; N.S., not significant. No. of animals in parenthesis.

type and mean red blood cell potassium concentrations.

All animals in a small experimental flock of Merino sheep which had been selected originally on the basis of red blood cell potassium concentrations were therefore examined along with a small flock of Southdowns. The results are shown in Table 2.

Low potassium (*A*) Merino sheep had a significantly higher mean red blood cell potassium concentration than low potassium (*AB*) animals ($P < 1·0$ per cent) or low potassium (*B*) animals ($P < 1·0$ per cent). Sufficient animals in the three hæmoglobin phenotypes were only available for the low potassium Merino, but other results show a similar trend—a trend which had been noted previously in relation to whole blood potassium concentrations[9].

The correlations between median corpuscular fragility and potassium type, and between potassium type and hæmoglobin type, are of interest in relation to the possible adaptive significance that the gene pairs controlling potassium and hæmoglobin types may have in some Australian environments[11,12].

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Human, Cow and Rabbit Native Fibrin Powders as Anticoagulants

The anticoagulant action of fibrin-coated surfaces on native blood and plasma has been demonstrated recently[1,2]. Since the fibrin-coating process is quite laborious[3], we tested the anti-coagulant effect of a fibrin surface by simply placing native plasma in contact with fibrin powder. It was realized that the contact of native plasma with surfaces foreign to blood known to initiate coagulation, such as glass, would have to be counteracted by a large enough fibrin surface. In a preceding investigation by Copley and Cliffton[4] so-called fraction I fibrin powder, prepared from a Cohn fraction I of bovine plasma by the addition of thrombin, was found to have an anti-coagulant action similar to that observed in glass tubes coated with bovine fraction I fibrin. Since the bovine fibrinogen (Armour) contains 40–50 per cent sodium citrate, it was not clear whether the latter was responsible for the anticoagulant effect of the fraction I fibrin powder. Therefore, work has been done on native plasma fibrin powder, prepared from native plasma without the addition of anticoagulants or coagulants.

Blood was drawn from the antecubital vein of healthy human subjects, the jugular vein of cows, or the rabbit's heart and immediately centrifuged for 10 min. at 5,000 r.p.m. at 0° C. The undiluted native plasma thus obtained was transferred to a glass tube and allowed to coagulate at room temperature, and the clot removed with a glass rod and incubated at 37° C. in a Petri dish to speed up retraction. The serum exuding from the clot was absorbed by filter paper every 10 min. for 1 hr., by which time clot retraction appeared to be complete. The clot was then transferred to another Petri dish and was allowed to dry in the incubator for about 21 hr. after which the fibrin was ground to a fine powder. 80 mgm. of the powder were mixed with 1 ml. of freshly prepared native plasma in a glass tube which was shaken by flicking it several times to ensure a thorough contact of the plasma with the powder. The tubes were incubated in a water-bath at 37° C. and observed for coagulation. As a rule, the plasma was kept in contact with the powder for 1 hr. and then was sepacated from the fibrin particles by low-speed centrifugation. Such so-called 'fibrin-contact plasma' was then further observed for clotting in glass tubes at 37° C. In all cases, regardless of the species used, coagulation never was found to occur for 7 or more hours. Thereafter, the plasma was stored in a refrigerator at 4° C. to counteract bacterial growth, and coagulation times were taken on successive days for periods up to several months. The initial contact of 1 hr. of rabbit native plasma with cow native fibrin powder did not result in coagulation for 98 days.

Table 1. Effect of Thrombin on Human, Cow and Rabbit Fibrin-Contact Plasma
(5 N.I.H. units thrombin per 0·1 ml. plasma)

Hours of contact with fibrin powder	Gelation times (sec.) of fibrin contact plasma at 37° C. Human native plasma exposed to human native fibrin	Rabbit native plasma exposed to rabbit native fibrin	Rabbit native plasma exposed to cow native fibrin
1	4·1	10·8	7·3
3	4·6	11·2	8·4
26	4·9	11·0	8·9

Whenever coagulation times were taken, the fibrin-contact plasma was tested for its coagulability by mixing 0·1 ml. with 0·1 ml. thrombin solution, containing 5 N.I.H. (National Institutes of Health) units, and timing the appearance of gel formation. As seen from Table 1, the thrombin coagulability of the fibrin-contact plasma does not change significantly when the native plasma is in contact with the fibrin for 1–26 hr. Human native plasma exposed to autologous native fibrin gives the shortest clotting or gelation times on the addition of thrombin, while this is not always the case with rabbit native plasma exposed to autologous native fibrin which exhibits longer clotting times. As can be seen, rabbit plasma exposed to cow native fibrin gives shorter clotting times with this thrombin test. The possibility of species-specific reactions and their variations is under investigation. Frequently we observed that ageing of fibrin-contact plasma was associated with a lengthening of the

thrombin clotting times. However, ageing had a similar effect on oxalate plasma of the same blood withdrawal used as a control.

Fibrin-contact plasma, prepared by using native fibrin from the three species, exhibited anticoagulant action. This was tested by mixing 2 vol. of fibrin-contact plasma with 1 vol. of native plasma, resulting in markedly prolonged clotting times or in no coagulation. Such mixtures always showed coagulation with the above thrombin test. When 2 vol. of native plasma were mixed with 1 vol. of fibrin-contact plasma, coagulation occurred, and the clotting times were only slightly longer than the controls of native plasma.

In contrast to fraction I fibrin powder, plasma exposed to native fibrin powder often exhibited decreases in fibrinogen content following incubation of 1 or more hr. regardless of the species used for the native fibrin. Such decreases amounted to 44 per cent for 2 hr. of contact when compared with the control of oxalate plasma from the same blood. It will have to be established whether the fibrinogen lost from the fibrin-contact plasma has been adsorbed to the native fibrin or whether it has been converted into fibrin. The use of native fibrin appears to differ also in another way from that of fraction I fibrin. By using the latter in both native plasma and whole blood, coagulation could be prevented; however, when native whole blood was exposed to native fibrin such an anticoagulant effect did not always result. Our findings appear to show that the use of fraction I fibrin introduces possible artefacts in contrast to that of native fibrin. The latter may well prove to have a more physiological anticoagulant action.

Our findings suggest that if the proposed endo-endothelial fibrin film[1,2,5] did exist, it might have an anticoagulant action. This might well aid in maintaining the fluidity of blood in the circulation. Outside some promising practical applications, our results necessitate alteration of certain views, generally believed to be responsible for the fluidity of circulating blood and the origin of thrombosis.

We are grateful to Dr. R. G. Macfarlane, Radcliffe Infirmary, Oxford, for helpful discussions, and to Mr. F. A. Glover, of the National Institute for Research in Dairying, University of Reading, for the preparation of cow native plasma fibrin.

The work was aided by grant *H*–4793 from the National Institutes of Health, Bethesda, Md.

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PATHOLOGY

Persistence of Motility in Bone-Marrow Cells from the Cadaver

A READILY available source of large quantities of bone marrow would be valuable for replacement therapy in such cases as radiation accidents. Cadaveric marrow may be suitable for this purpose as the cells are believed to remain viable for several hours after death[1], although no criteria of viability are given. The present work was undertaken to determine the persistence of viability of marrow cells by investigating their motility *in vitro* after somatic death.

Sternal marrow (0·1–0·2 ml.) was obtained by normal puncture technique from fifty human subjects at various times after death. Bodies were refrigerated about 2 hr. after death. The range of age was 33–89 although the great majority were more than 50 years old and the ratio of males to females was approximately 2 : 1. An occasional sample was taken within 2 hr. of death; increasing difficulty was often experienced the longer after death the attempt was made.

Most leucocytes of the bone marrow exhibit some degree of motility although this is greatest in the neutrophil polymorphonuclear[2], and the present results are based for the most part on the motility of this cell. Motility was considered to be present if a cell was observed to move a distance of approximately its own diameter or more during a 5-min. search of the preparation.

For examination of the specimen a drop of marrow was expelled on to a chemically cleaned glass slide, a coverslip applied and the edge sealed with soft paraffin. The remaining marrow was mixed with bovine albumin (5 per cent) in physiological saline and used to prepare stained films for differential counting[3]. This is important because for practical purposes polymorphs represent the only motile cells of the marrow and any notable reduction of their number clearly reduces the chances of observing motility. The sealed preparation, on a warm stage at 37° C., was examined for motility with the 1/6 objective. A 5-min. search usually entailed the examination of about twelve different fields.

In a preliminary examination of living subjects 12 out of 14 samples from routine diagnostic sternal marrow biopsies showed motility. Of the 2 negatives one was a case of lymphocytic leukæmia, the other a case of myelosclerosis.

In the post-mortem investigation, 50 results were assessed as satisfactory for analysis (Table 1). Cases were excluded from analysis because of myeloproliferative or aplastic disorders, failure of refrigeration of the body or unavailability of temperature records within 24 hr. of death. The presence of pyrexia before death, that is a temperature of more than 37° C. at some stage during the terminal 24 hr., reduced motility and such cases are recorded separately in Table 1. Thirty-four of the fifty cases showed motility. This was invariably present less than 5 hr. after death and up to 20 hr. in apyrexial subjects and absent in all subjects after 40 hr. Oscillation of the intra-leucocytic granules frequently persisted after detectable motility had ceased. McLeod and McLeod[4] believe that such oscillation is a criterion of the viability of leucocytes. Whitby and Britton[2], while by inference agreeing that cells exhibiting these properties are living, state that the oscillation is a preliminary to cellular death and the present investigation supports this view. Monocytic and lymphocytic motility exists[2,5,6], but was considered unimportant because of their small numbers and sluggish movement.

Table 1

		Hours after death 0–20	> 20
a	Motile	18	3
	Non-motile	0	5
b	Motile	9	4
	Non-motile	3	8

a, Apyrexial; *b*, pyrexial.

Cellular viability in the bone marrow of the refrigerated cadaver persists for 20 hr. or more after death. A shortening of the period of motility by ante-mortem pyrexia is in accord with Jeanneret's[7] finding that in pyrexial human subjects degeneration of cells in the stained film of post-mortem marrow occurs earlier than in the apyrexial subject. It is uncertain whether this phenomenon is attributable to a common factor, for example toxæmia, which causes pyrexia and hastens cellular death, or to the pyrexia alone increasing the metabolic rate of the cell with quicker exhaustion of its energy potential.

The clinical inference from these leucocyte motility investigations is that cells may remain transplantable longer than hitherto supposed. The technique for studying the motility is a simple one which should be useful in other ways, as for example in the examination of possible cytotoxic effects of anti-leucocytic sera.

I wish to thank Dr. J. F. Wilson for the idea from which this work developed as well as Prof. J. S. Young, Dr. R. C. Nairn and Mr. W. Brass for their interest and advice.

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Effect of Phagocytosis on the Lysis of *Staphylococcus aureus* (Type 81) by Bacteriophage

Certain investigations on animals have suggested the efficacy of treatment of bacterial infections with specific bacteriophage. Dubos *et al.*[1] demonstrated the therapeutic effect of *Shigella dysenteriae* phage in experimental infections in mice. Morton and Engley[2] showed a prophylactic, as well as therapeutic, effect in mice infected with *Shigella dysenteriae*. Recently, Muir and Blakemore[3] protected 70 per cent of mice lethally infected with *Staphylococcus aureus* type 80/81 with high-titred specific phage given simultaneously with the bacteria.

Work on the use of bacteriophage *in vivo* is necessarily complicated by a variety of factors, one of which is the role of phagocytes. This report will present evidence to indicate that *S. aureus* is phagocytized *in vitro* by rabbit monocytes and polymorphonuclear leucocytes, and that bacteria released from the phagocytes are susceptible to subsequent lysis by specific bacteriophage.

Staphylococcus aureus, propagating strain 81, was grown and maintained on tryptacase soybean broth (TSB). Type 81 staphylococcal bacteriophage was propagated on *S. aureus* strain 81 in the same broth, filtered through UF fritted glass filters and was stored at 4° C. Polymorphonuclear leucocytes (PMN) were obtained from normal rabbits by injecting 100 ml. of sterile 0·85 per cent sodium chloride intraperitoneally, followed by a similar injection 18 hr. later. Rabbits were killed about 3 hr. after the second injection either by bleeding or by a blow on the back of the head and the peritoneal cavities were opened aseptically. Peritoneal fluid was removed into sterile 0·85 per cent saline containing about 10γ of heparin per ml. Monocytes (MN) were obtained from rabbits given 100 ml. of sterile heavy mineral oil intraperitoneally and killed three days later. Both MN and PMN were washed three times with sterile Hanks's solution and collected after centrifugation at 1,000 r.p.m. for 5 min. at 4° C. By these methods about 75 per cent of the collected cell population were of the desired type.

In the experiments reported here, 5 ml. of an 18 hr. culture of *S. aureus* (about 5×10^{10} bacteria/ml.) were mixed with an equal volume of either PMN or MN suspended in medium 199 to contain 2×10^6 cells/ml. These mixtures were incubated in a roller drum revolving at 15 r.p.h. at 37° C. for 45 min. Following incubation, the mixtures were immediately chilled in an ice-bath and all subsequent washings of the cells were carried out at 4° C. The original cell bacteria mixtures were washed five times with Hanks's solution and the cells resuspended at 1×10^6 cells per ml. in medium 199. One of these suspensions was taken as the zero time sample. The tubes were tightly stoppered and returned to the roller drum. At intervals one tube was removed from the roller drum and washed as described. The final cell pellet was lysed in 1·5 ml. of sterile distilled water, releasing intracellular bacteria, and isotonicity was restored with an equal volume of sterile 1·6 per cent sodium chloride. Ten-fold dilutions were made in TSB in 1·0-ml. amounts. To one-half of each dilution 0·5 ml. of phage type 81 (RTD = 10^{-4}) was added. The same volume of TSB was added to the other half of each dilution. These mixtures were incubated at 37° C. for 10 min. and were poured on to fresh tryptacase soybean agar plates. All plates were incubated at 37° C. overnight and colony counts carried out the following day.

The colonies present on each control (C) plate and each test or phage-treated plate (T) at each time-interval and at each dilution were counted. In three experiments with PMN and two with MN from five different rabbits, the control colony count at each time-interval reflected the ten-fold dilutions made. The colony counts given in Table 1 (PMN) and Table 2 (MN) represent geometric means of the three ten-fold dilutions using cells from five rabbits at each time interval. The T/C ratios were calculated from the geometric means at each time-interval. The T/C ratio for both PMN and MN reached its peak at about the same time the intracellular bacterial count was lowest. The number of bacteria not lysed by the phage was quite constant within the time-limits of the experiments, while the number of intracellular bacteria not exposed to phage decreased steadily for about 1·5 hr. in PMN, then increased. MN, on the other hand, kept the number of viable intracellular

Table 1. T/C Ratios and Geometric Means of Colony Counts in PMN from Three Rabbits

Hr.	Colony count T*	Colony count C†	T/C
0	2	475	$4{\cdot}2 \times 10^{-3}$
1	1·5	69	$2{\cdot}2 \times 10^{-2}$
1·5	1·4	25	$5{\cdot}6 \times 10^{-2}$
2	1·6	46	$3{\cdot}5 \times 10^{-2}$
2·5	2·6	61	$4{\cdot}2 \times 10^{-2}$

T*, Phage treated; C†, no phage treatment.

Table 2. T/C Ratios and Geometric Means of Colony Counts in MN from Two Rabbits

Hr.	Colony count T*	Colony count C†	T/C
0	1	74	$1{\cdot}4 \times 10^{-2}$
1	1·5	13	$1{\cdot}1 \times 10^{-1}$
1·5	1·1	8	$1{\cdot}4 \times 10^{-1}$
2	1·1	7	$1{\cdot}6 \times 10^{-1}$
2·5	1·2	7	$1{\cdot}7 \times 10^{-1}$

T*, Phage treated; C†, no phage treatment.

bacteria at a constant low level for at least 1 hr. longer. Microscopic examination revealed no viable phagocytes in either system after 24 hr.

Bacteria which were not lysed by the phage after passage through either PMN or MN were later plated and incubated at 37° C. for 18 hr. When these bacteria were exposed to phage 81, complete lysis occurred. This indicates that after passage through either PMN or MN, *S. aureus* was unaffected in its susceptibility to lysis by phage 81.

The meaning of the *T*/*C* ratio is perhaps best explained by the *T* and *C* columns in Tables 1 and 2. The number of viable bacteria in the phage-treated (*T*) columns was nearly constant in five different experiments. If phagocytosis caused an increase in the number of phage-resistant bacteria, the *T*/*C* ratio should approach unity when the control values fell. However, the approach of the *T*/*C* ratio toward unity was entirely due to a decrease in control counts and not to an increase in test counts. Thus the *T*/*C* ratio also becomes a measure of the bactericidal activity of the phagocytes and the higher the *T*/*C* ratio, the greater the bactericidal activity.

Our results appear to be in partial agreement with the findings of other workers[4,5]. Cohn and Morse[4] found that *S. aureus* was not readily phagocytized in normal rabbit serum unless the multiplicity was ten bacteria/leucocyte. However, *S. aureus* was killed intra-cellularly only in the presence of specific immune serum. Mackaness[6] reported similar findings using rabbit macrophages (monocytes). Kapral and Shayegani[5] demonstrated phagocytosis by rabbit MN and PMN at a multiplicity of 25 or 50 : 1, but reported no inactivation of intracellular bacteria. Our results suggest that the intracellular inactivation of *S. aureus* was accomplished by both PMN and MN to the point where the number of extracellular bacteria reached a critical value[5], and the cells became overwhelmed by bacteria. The increase in viable intracellular bacteria probably does not represent multiplication, but may be due to additional phagocytosis[5].

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RADIOBIOLOGY

Induction of Tumours in Mice with Tritiated Thymidine

We wish to record preliminary results of an experiment, in which we are investigating the possibility of inducing tumours in mice with tritiated thymidine. The β-particles from tritium incorporated into deoxyribonucleic acid (DNA) from ^{3}H-thymidine produce an irradiation which primarily affects the cell nucleus, since about 90 per cent of the energy of these particles is dissipated within the nucleus. This procedure, therefore, enables one to distinguish in long-range experiments the results of nuclear damage from that caused by irradiation of whole cells and tissues, and to study the importance of nuclear damage in radiation carcinogenesis. It is desirable to do this, since there are many indications that alterations in deoxyribonucleic acid are involved in carcinogenesis in general. It is also commonly assumed that the tumour-inducing properties of ionizing radiations are mediated through changes in the nucleus, but there is no proof of this assumption.

Table 1. Incidence of Tumours in CAF_1 Mice after a Single Intraperitoneal Injection of ^{3}H-Thymidine

Groups	Dose-level	No. of animals	Age at time of injection (months)	Age at present (months)	No. of deaths	No. of animals with tumours
1	1·0 μc./gm.	100	new-born (1-day)	17	8	0
2	1·0 μc./gm.	100	2	19	23	8
3	1·0 μc./gm.	98	6–7	23–24	34	16
4	Control	170	new-born (1 day)	17	9	2
5	Control	99	3–4	20–21	21	4

Our experiment has now been in progress for 17 months. It involves more groups of animals than those mentioned here. The mortality among the experimental animals has been remarkably low and the capacity of tritiated thymidine to induce tumours under these conditions has not been spectacular. This is of some interest in itself. Nevertheless, although the total number of tumours is small, there is evidence that the death-rate from tumours is significantly higher among the exposed than among the control animals.

Three groups of male and female CAF_1 mice received a single intraperitoneal injection of ^{3}H-thymidine, with a specific activity of 360 mc./m.mole (New England Nuclear Corporation, Boston, Mass.), at a dose-level of 1·0 μc./gm. of body-weight. The groups differed from one another in age at the time of injection as follows (Table 1): Group 1 consisted of new-born animals less than 24 hr. old; the animals of Group 2 were 2 months old and those of Group 3 6–7 months old. Groups 4 and 5 served as controls. Group 4 consisted of new-born animals and those of Group 5 were 3–4 months old. The controls were injected either with non-radioactive thymidine or with saline solution. The animals were permitted to live out their life-span. The diagnosis was verified in each instance by histological examination.

The following types of malignant tumours have been observed among the experimental animals: 6, thymic lymphoma; 9, non-thymic, disseminated lymphoma; 2, salivary gland tumour; 2, carcinoma of the skin; 5, carcinoma of the lung; 1, carcinoma of the liver (hepatocellular). The tumours were distributed equally among males and females. The six tumours among the control animals were: 5 non-thymic lymphomas and 1 carcinoma of the lung. In animals without tumours, death could be attributed to a variety of causes, including pneumonia and intestinal infections. It should be mentioned that no lung adenomata have been observed so far, either in the control or in the experimental animals.

These results indicate that, at this dose-level, tritiated thymidine is a carcinogen in mice. This conclusion is supported by a similar finding in another group of 100 animals in the same experiment that were 12–14 months old at the time of injection. Of these animals, 84 are now dead and 43 of them have died with tumours. Although we do not at the moment have age-specific controls for this group of animals (and hence we have not listed them in Table 1), a tumour incidence of more than 50 per cent is considerably higher than that ordinarily seen in control animals of this strain and age in our Laboratory.

XUM

No results are available as yet in another part of this experiment, in which we are studying the comparative effectiveness in the induction of tumours by ^{14}C-thymidine with its more penetrating β-rays.

The observations on induction of tumours by tritium-labelled thymidine are being reported now not only because they are of interest in connexion with the question of nuclear damage in radiation carcinogenesis, but also because they lend weight to the evidence that tritiated thymidine can produce significant changes in mammalian cells. Such changes have included short-term inhibition of growth[1,2], developmental and chromosome abnormalities[3,4], as well as cytogenic aberrations, as shown by an increased frequency of mutations in *Drosophila*[5]. The observations in the present experiment also tend to support the opinion of Oliver and Lajtha[6], who have given warning of the hazards involved in using tritium as deoxyribonucleic acid label in man. They pointed out the possibility of mutagenic effects of the radiation on those cells that have a long life-span and several mitotic cycles.

This work was carried out under the auspices of the U.S. Atomic Energy Commission and supported by U.S.A.E.C. contract No. *AT* (II–I)–809.

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Mouse Lymphoma Cells with Different Radiosensitivities

FISCHER[1] devised a medium in which murine leukæmia cells (*L* 5178 *Y*) can be grown in free culture starting from a single cell. These cells have retained their strain specificity and they will grow only in *DBA*/2 mice where they give rise to ascites and solid tumours. A logarithmically growing culture was obtained 24 hr. after transfer of the ascites cells.

We have exposed cultures to 220 kVp. X-rays and observed that cells changed their radiosensitivity on being maintained continuously in culture. Fig. 1 shows the effect of irradiation on cultures that had been started from cells obtained six to ten days earlier from a mouse. Cells that had been kept going for six months continuously in culture—transfer to new medium being made twice a week—were very much more radiosensitive due to the appearance of a radiosensitive mutant. Sensitive strains were isolated by cloning from a single cell[2] and their response to radiation is shown in Fig. 2.

The effect of radiomimetic alkylating agents on these lymphoma cells has been described[3] and their response to radiation is similar. With doses up to the LD_{99}, most of the cells divide at least once and degenerate after the second day when they have grown to double their volume as measured with a Coulter cell counter. Before lysing, 10–20 per cent of the cells grow into giants having ten to fifty times the normal volume. With higher doses of radiation (that is, greater than 500 r. for the resistant strain and 200 r. for the sensitive strain) an increasing proportion of cells degenerate without having undergone division. Cells that have retained their capacity for indefinite multiplication give rise to the new population that grows up from the cultures. The fraction of 'survivors' can be calculated by extrapolation[3] and the dose response curves shown in Fig. 3 have been obtained in this way. If radiation induces a delay in mitosis which extends over several hours then the number of survivors obtained by the extrapolation method would be an underestimate. While this is improbable for reasons which we have given[3], the method was checked by determining the dilution from which an irradiated culture grows up and which must therefore contain at least one viable cell—the cloning efficiency from one cell being between 70–80 per cent[2]. The number of 'survivors' computed in this way agreed with doses up to 300 r. with the values obtained by the extrapolation method.

We have indications that mutants with varying degrees of radiosensitivity arise during growth in culture, but we have not found any strain more resistant than that carried by passage through animals. The sensitive strain divides in culture about 25 per cent more rapidly than the insensitive (compare control curves in Figs. 1 and 2) and this presumably gives them a proliferative advantage so that they establish themselves *in vitro* on continued culture. The sensitive cells have retained their host specificity for *DBA*/2 mice, but they grow less rapidly in animals than the resistant and give rise more frequently to solid tumours.

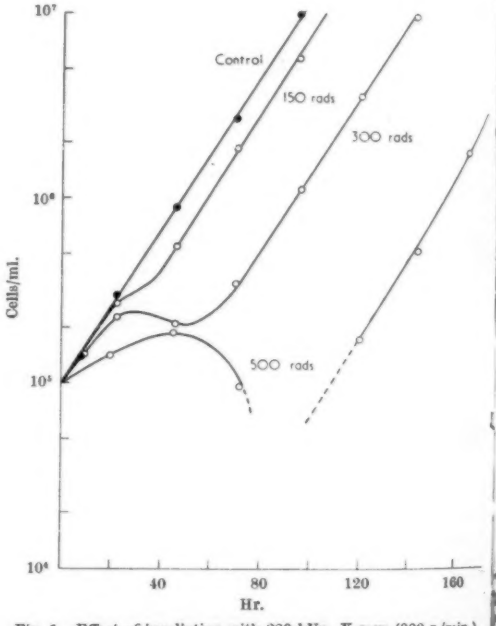

Fig. 1. Effect of irradiation with 220 kVp. X-rays (900 r./min.) on lymphoma cells in a culture which was fully aerated. The culture was diluted before reaching 10^7 cells./ml. and counted in a hæmocytometer.
Cells transferred to culture from the ascites fluid of a mouse ten days earlier; referred to as a resistant strain

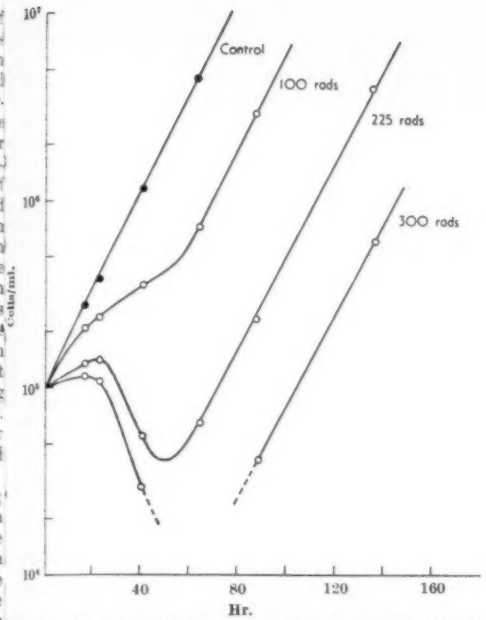

Fig. 2. Effect of irradiation with 220 kVp. X-rays (900 r./min.) on lymphoma cells in a culture which was fully aerated. The culture was diluted before reaching 10^7 cells/ml. and counted in a hæmocytometer.
A cloned derivative from cells continuously maintained in culture for six months; referred to as sensitive strain

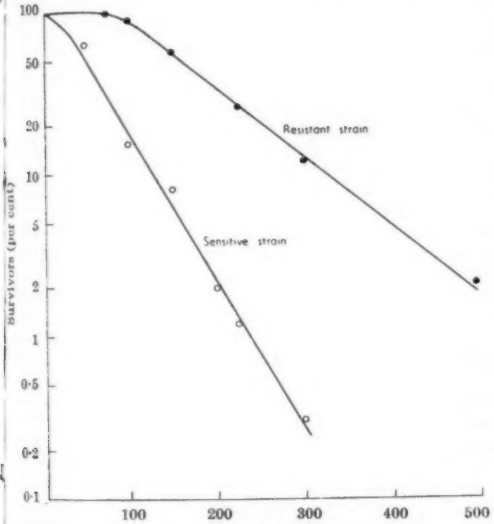

Fig. 3. Fraction of cells capable of normal growth as a function of radiation dose

The average volume, as measured with a Coulter counter, of the sensitive cells is some 30 per cent greater than that of the resistant cells but the content of deoxyribonucleic acid, determined by Burton's method, is the same for both. In neither type of cell, when taken from culture or from animals, could any viruses be seen in the electron microscope.

These experiments demonstrate that the radiosensitivity of mammalian cells *in vitro* can change, presumably by a mutation, and suggest that deductions concerning the radiosensitivity of cells *in vivo* from *in vitro* studies are liable to serious error. Fischer[4] has shown a similar phenomenon with regard to resistance to folic acid antagonists.

This work was supported by a grant from the International Atomic Energy Agency and by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Effects of Iodoacetic Acid on the Incorporation of Phosphorus-32 into the Deoxyribonucleic Acid of some Radiosensitive Organs in Rats exposed to X-Rays

It is now known that iodoacetic acid is able to sensitize mice[1,2] and rats[3] to ionizing radiations as well as cultured mammalian cells[4] and bacteria (Bridges, B. A., personal communication).

In a recent paper[5] we presented evidence that iodoacetic acid markedly inhibits the uptake of phosphorus-32 into the deoxyribonucleic acid (DNA) of some radiosensitive organs and of the regenerating liver in the rat. This effect is similar to that shown by ionizing radiations that inhibit the incorporation of ^{32}P, as well as of many labelled precursors, into DNA of dividing irradiated cells[6]. Considering this common interference with DNA synthesis, we have undertaken work on the effect of the combined treatment with iodoacetic acid and X-rays on ^{32}P uptake into DNA of rat thymus, spleen and intestine.

Laboratory bred male albino rats (70–90 gm.) of Wistar Glaxo strain were used. Lots of 16 animals, subdivided into 4 groups of 4, were used in each experiment, and treated according to the schedule reported in Tables 1 and 2.

Iodoacetic acid at a dose of 40 mgm./kgm. was administered intravenously. This dose, as already indicated in a previous paper[5], is definitely sub-lethal. X-irradiation was performed with a Picker instrument operating at 220 kV., 15 m.amp., with an added filtration of 0·5 mm. copper and 1 mm. aluminium, at a dose rate of 26 r./min., for a total dose of 150 r.

When the animals were subjected to a combined treatment, irradiation was performed 30 min. after the administration of iodoacetic acid.

At a dose of 25 μc./rat ^{32}P was injected intramuscularly immediately after irradiation. Non-irradiated rats were given ^{32}P 36 min. after the iodoacetate treatment. The animals were killed with ether 2 hr. after the injection of ^{32}P, and the thymus, spleen and 15 cm. of the small intestine, just distal

Table 1. Corrected Specific Activity of DNA* extracted from the Spleen, the Intestine and the Thymus of Normal and Treated Animals 2 hr. After the Administration of 25 μc. ^{32}P/Rat. (Mean ± Standard Error of the Mean)†

	Controls	Iodoacetic acid (40 mgm./kgm.)	X-rays (150 r.)	Iodoacetic acid (40 mgm./kgm.) X-rays (150 r.)
		(21 per cent)‡	(46 per cent)	(56 per cent)
Spleen	4·44 ± 0·76	3·52 ± 0·63	2·40 ± 0·34	1·94 ± 0·36
		(12 per cent)	(5 per cent)	(24 per cent)
Intestine	2·16 ± 0·19	1·90 ± 0·30	2·05 ± 0·16	1·64 ± 0·16
		(23 per cent)	(28 per cent)	(50 per cent)
Thymus	3·64 ± 0·36	2·79 ± 0·35	2·61 ± 0·27	1·81 ± 0·14
			$t = 2·606$;	$0·05 > P > 0·02$

* Corrected specific activity of DNA = $\frac{\text{specific activity DNA (e.p.m./μgm. phosphorus)}}{\text{specific activity of blood orthophosphate (e.p.m./μgm. phosphorus)}} \times 100$

† Each figure is an average of five values obtained from the pooled organs of four animals.

‡ Figures in brackets represent percentage of inhibition.

to the pylorus, were rapidly removed. The organs of the four animals of each group were pooled for the extraction of DNA. All successive steps have been described in our previous paper[5].

Table 1 contains the values of the corrected specific activity of DNA extracted from thymus, spleen and intestine of the experimental animals. These values show an inhibiting effect following treatment with iodoacetic acid, as already described. The results, moreover, confirm the well-known inhibitory effect of radiation on ^{32}P uptake into DNA from lymphoid organs, whereas the effect is not yet detectable for the intestine at the dose-level employed. Finally they show that after combined treatment, the inhibitory effect nearly corresponds to the sum of the effects obtained by single treatments.

Experiments in which ^{32}P was injected 4 hr. after irradiation have also been carried out. The results are reported in Table 2. In this case we considered only thymus and intestine. These experiments were undertaken because the inhibition by irradiation of the incorporation of labelled precursors into DNA of radiosensitive organs reaches higher values after this time interval[7].

Table 2. Corrected Specific Activity of DNA extracted from the Intestine and the Thymus of Normal and Treated Animals 2 hr. After the Administration of 25 μc. ^{32}P/Rat (X-ray Treatment 4 hr. Before ^{32}P)

	Controls	Iodoacetic acid (40 mgm./kgm.)	X-rays (150 r.)	Iodoacetic acid (40 mgm./kgm.) X-rays (150 r.)
		(12 per cent)†	(46 per cent)	(49 per cent)
Intestine	2·43*	2·14	1·31	1·24
		(9 per cent)	(63 per cent)	(73 per cent)
Thymus	3·09	2·82	1·13	0·82

* Each figure is an average of three values obtained from the pooled organs of four animals.

† Figures in brackets represent percentage of inhibition.

It will be noted that the effect of irradiation is much more marked at 4 hr. than at 2 hr., as expected; whereas the effect of iodoacetate is clearly lower. The response to the combined treatment in this case also nearly corresponds to the sum of the effects obtained after single treatments.

In conclusion the results reported in the present paper seem to indicate that (*a*), the combined treatment with iodoacetate and X-rays, at the doses used in our experiments, causes an inhibition of ^{32}P uptake into DNA corresponding nearly to sum of the inhibitions obtained by single treatments; (*b*), the effect of iodoacetate decreases significantly a few hr. after the administration of this substance, whereas at the same time interval the irradiation effect increases.

A possible interpretation of the results could be that the effect of iodoacetate on DNA synthesis combining with the effect of radiation may increase the total cellular damage in radiosensitive tissues.

In discussing the present results it must be taken into account that, with the doses of X-rays used in our experiments, it is possible that the reduction in DNA synthesis is the result of temporary mitotic delay and not of cell-death, whereas the sensitizing effect of iodoacetic acid has been established for cell-death. Another point to be considered is that the effect of iodoacetic acid seems to be reversible in a short time. It is not yet known whether the increased inhibition of DNA synthesis, induced by iodoacetic acid in combination with low doses of X-rays, can really affect the final cellular damage in radiosensitive tissues.

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Protection by Inorganic Condensed Phosphates against the Lethal Effects of X-rays

A radioprotective effect of adenosine triphosphate has been described by Langendorff *et al.*[1], but the inorganic condensed phosphates do not appear to have been studied previously in this connexion. We have found that sodium pyrophosphate, the linear tripolyphosphate and the cyclic trimeta- and tetrametaphosphates markedly reduce the mortality in mice due to X-rays when injected intraperitoneally in neutral solution, shortly before exposure.

Female mice, weighing 20–25 gm., of *R* stock (a cross between *PCT* female and *C3H* × 101 male) were used at the age of 14 weeks. In a typical experiment 50 mice, randomized into groups of 5, were irradiated in partitioned boxes as follows: 250 kV., 15 m.amp., H.V.L. 1·2 mm. copper, distance to underside of box 60 cm., average dose-rate 73·6 r./min. Ten mice were used on each of 4 protective treatments, with 10 saline-treated controls. All 5 treatments were represented in each group during irradiation and the subsequent period of observation. Survival at 30 days was taken as the measure of protective effect. In a series of experiments on this pattern 49 saline-treated control animals died after 1,025 r., out of a total of 53 used. Thus the overall control mortality was 92 per cent, and this can be used for statistical tests with the tables of Fisher and Yates[2]; but the mouse population may change its radiosensitivity and a safer though more severe test, using only the simultaneous controls, is made possible by Fisher's exact 2 × 2 contingency function as tabulated by Mainland, Herrera and Sutcliffe[3]. The results shown in Table 1 were submitted to both tests as indicated.

Since trimetaphosphate at 15 m.moles/kgm. protected 100 per cent against 1,025 r. a further experiment was done to estimate to what level it raised the radiation LD_{50}, and to compare it in this respect with *S*,β-aminoethylisothiuronium bromide hydrobromide (AET) at 1 m.mole/kgm., the highest safe dose for mice. Both were given intraperitoneally 10 min.

Table 1. Protective Effects of Inorganic Condensed Phosphates against 1,025 r. of X-rays

Compound	Dose (m.moles/kgm.)	Time of injection (min. before irradiation)	No. of mice surviving: No. used	Exp.	Controls (saline)
$Na_3P_3O_{9}.10H_2O$	0·75*	10	6/10†		1/10
..	0·50	10	2/10		1/10
$Na_3P_3O_{10}.6H_2O$	1·00*	10	14/20‡		0/20
..	0·75	10	3/10		0/10
..	1·00	30	8/10‡		0/10
..	1·00	60	2/10		0/10
$(NaPO_3)_6.6H_2O$	15*	10	10/10‡		1/10
..	9	10	1/10		
$(NaPO_3)_4.4H_2O$	2·4*	10	8/10‡		

* These doses resulted in no deaths ascribable to toxicity. At higher dose-levels some deaths occurred shortly after irradiation, as a result of potentiation of toxicity by X-rays.
† Significant ($P < 0.005$) assuming 8 per cent control mortality (ref. 2).
‡ Also significant ($P < 0.01$) by 2 × 2 contingency test using only internal controls (ref. 3).

Table 2. Protective Effects of Trimetaphosphate and AET

X-ray dose (r.)	No. of mice surviving at 30 days out of: 4 given trimetaphosphate	4 given AET	2 given saline
1,050	3	4	0
1,100	2	4	0
1,150	3	4	0
1,200	0	4	0
1,250	1	4	0
1,300	1	0	0
1 350	0	3	0

before irradiation. The results are given in Table 2. From these the normal-logarithmic maximum-likelihood estimate of the LD_{50} (by the iteration procedure of Cornfield and Mantel[4]) is 1,133 (1,059–1,213) r. for trimetaphosphate and 1,325 (1,265–1,387) r. for AET. The figures in brackets are 5 per cent fiducial limits.

Thus trimetaphosphate is significantly less protective than AET, but its lower fiducial limit, 1,059 r., is well above the LD_{50} of uninjected mice of this strain, which, though not measured simultaneously, is known to be about 860 r. There are several conceivable reasons for the protective activity of condensed phosphates, which we are investigating.

We wish to thank Mrs. D. M. R. Lowman for able technical assistance, Mr. M. J. Corp for irradiations and Dr. D. R. Peck, of Albright and Wilson, Ltd., for his interest and for generous gifts of pure chemicals.

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BIOLOGY

'Bound' Gibberellin in Mature Runner Bean Seeds

Radley[1] has mentioned the possibility that gibberellins occur in the tissues of higher plants in both 'free' (readily extractable) and 'bound' forms. By analogy with plant-growth regulating substances of the auxin type, the hypothetical 'bound' gibberellins might be liberated by the hydrolysis of proteins of the plants being investigated. This communication describes one of several experiments in which more 'gibberellin-like' activity was detected in hydrolysed than in non-hydrolysed extracts. Radley[1] has observed that the level of readily extractable gibberellin of the seeds of the runner bean (*Phaseolus multiflorus*) falls as the seeds mature. One could visualize that the gibberellin is becoming 'bound' in some way as maturation proceeds, and because of this possibility seeds of the runner bean were chosen for investigation.

Mature runner bean seeds, mean length 28 mm., were divided into three samples, each of 120 gm. Sample *A* was homogenized in 500 ml. of a mixture of equal parts of acetone and water. (A mixture of acetone and water has been used as a primary solvent for the extraction of gibberellins from plant tissue by several workers, for example Phinney *et al.*[2], Bünsow *et al.*[3], Lang[4], Lona[5], Murakami[6], and Nickell[7].) The extract was filtered through cheese cloth, centrifuged, and the acetone evaporated *in vacuo* to leave an aqueous residue which was adjusted to 500 ml. with distilled water.

Sample *B* was homogenized in 500 ml. of phosphate buffer, *p*H 6·2, made by dissolving 136 gm. of potassium dihydrogen orthophosphate and 24 gm. of potassium hydroxide per litre of water. (This buffer has been used by Borrow *et al.*[8] as a solvent at one stage in the isolation of gibberellic acid from fungal cultures, and by Radley[1] as a solvent at one stage in experiments demonstrating the presence of gibberellin-like substances in higher plants, but it was not used as a primary extracting solvent.) The extract was filtered and centrifuged.

Sample *C* was homogenized in 500 ml. of phosphate buffer, *p*H 6·2, and the extract filtered and centrifuged. 4·0 gm. of crude ficin (L. Light and Co., Ltd.) and 6·0 mgm. of cysteine were added to the extract, which was then stored at 30° C. for 12 hr. At the end of this time the extract was again centrifuged.

The three extracts were purified further in the same way: each was adjusted to *p*H 2·8–3·0 using *N* hydrochloric acid, and extracted three times with 400 ml. of ethyl acetate. The lower (aqueous) phase was discarded and the bulked ethyl acetate-rich extract reduced in volume to 50 ml. *in vacuo* at a temperature not exceeding 30° C. The extract so obtained was adjusted to *p*H 2·8–3·0 using *N* hydrochloric acid, and extracted three times with an equal volume of ethyl acetate. The bulked ethyl acetate-rich extract was reduced in volume and placed as a line across a 23-cm.-wide strip of acid-washed Whatman No. 3 chromatography paper. The chromatogram was run using a descending technique, and as a solvent the upper phase of a mixture of *n*-butanol/1·5 *N* ammonium hydroxide (3 : 1). Gibberellin A_1 (which is known to occur in the runner bean[9]) and gibberellic acid run with an R_F in the region 0·2–0·3 in this solvent system (see, for example, refs. 2, 10). When the solvent front had moved a convenient distance down the paper, some 25 cm. from the start line, each chromatogram was allowed to dry at room temperature and cut into transverse strips, each strip being in width one-tenth of the distance between the start line and the solvent front. Each strip was eluted with approximately 30 ml. of acetone in an apparatus designed by Canny[11]. The acetone was evaporated, and the dry residue stored in a deep freeze ready for assay.

Dwarf pea seedlings (var. Meteor) were used as assay plants in a technique essentially similar to that already described[10]. At the time of assay each residue was taken up in 0·8 ml. of 30 per cent ethanol,

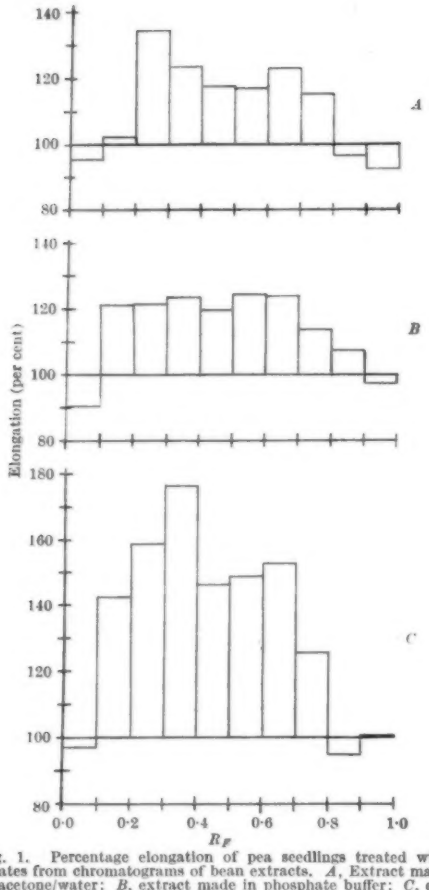

Fig. 1. Percentage elongation of pea seedlings treated with eluates from chromatograms of bean extracts. *A*, Extract made in acetone/water; *B*, extract made in phosphate buffer; *C*, extract made in phosphate buffer and hydrolysed with ficin

and a 0·004 ml. droplet of this solution was placed on each assay plant. A small puncture was made in the stem of each plant to aid penetration. Ten plants were used for each solution being assayed. Eluates from corresponding R_F values of the chromatograms of the three extracts were assayed in the same batch of assay plants.

Fig. 1 shows the mean elongation of the measured internodes of the treated plants expressed as a percentage of the corresponding internodes of the controls. It will be seen that there is considerably more activity in the hydrolysed than in either of the non-hydrolysed extracts. Activity is in each case spread over much of the chromatogram, but two zones are in evidence—one at R_F 0·2–0·4, the other at 0·6–0·7. By comparing the responses brought about by the extracts with those induced by known amounts of gibberellic acid, the total activities of the extracts were estimated as *A*, 0·04, *B*, 0·04, and *C*, 0·23 μgm. of 'gibberellic acid equivalents' per gram fresh weight of extracted material. That is, there is about six times as much activity in the hydrolysed extract. The gibberellins detected here may very well differ in their biological activities from the activity of gibberellic acid, so that the above figures should not be taken to indicate the actual levels of gibberellin in the tissue.

Other extracts of mature runner beans have shown increased activity due to enzyme hydrolysis, while extracts of young beans have in general shown no such increase. Negligible activity was detected in an extract subjected to alkaline hydrolysis. In all cases buffer has proved to be at least as effective an extracting solvent as a mixture of acetone and water.

I am indebted to Dr. M. J. Canny for his interest and advice during the course of these experiments.

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Gibberellic Acid and Chlorophyll Content of Leaves of Meteor Peas

The present investigation was to determine whether the effect of gibberellic acid in causing chlorosis was due to an interference in chlorophyll formation or to some secondary effect[1]. *Pisum sativum* var. Meteor were grown in the field in a block arrangement, and alternate rows were treated with gibberellic acid (*Y.F.* 4961, Plant Protection, Ltd.). Gibberellic acid diluted to 100 p.p.m. with water was sprayed on the upper surface of the plant 10 days after emergence.

Three days after spraying slight chlorosis of the third leaves was apparent. Chlorosis increased in intensity in leaves expanding afterwards, but no change in colour was observed in the first and second leaves. The internodes from the second upwards showed a 180–250 per cent increase in length over the controls. Measurement of the length and breadth of a random sample of leaves of control and treated plants was made 7 and 14 days after spraying. The total number of cells[2], chlorophyll content[3], and the fresh and dry weight of a finite area of leaf were measured for disks of known area punched from a similar position 8 and 15 days after spraying. Measurements were also made of individual palisade cells in a similar region from which the disks were punched, and the depth of the sections measured to find the thickness of leaf in this area.

Measurements of the leaf dimensions showed that those of treated plants, with the exception of the oldest leaf, were longer than those of the controls, but of similar breadth. Palisade cells in treated material tended to be broader and shorter in length. The results given in Table 1 show that total chlorophyll tended to be slightly less in treated leaves expanded after spraying. However, chlorophyll content expressed in terms of dry weight, fresh weight, or weight/cell shows no such decrease. Cell counts show that treatment with gibberellic acid resulted in a significant decrease in the number of cells per unit leaf area in leaves not fully expanded at the time of spraying. This decrease is related to the decreased thickness of the leaf.

Table 1

Leaf No.	Total chlorophyll (mgm./90 mm.²)		Chlorophyll gm./100 gm. fresh weight		Chlorophyll gm./100 gm. dry weight		Chlorophyll per cell, μgm. × 10^{-5}		Ratio chlorophyll a/b		Leaf thickness (μ)		No. of cells/ 90 mm.² × 10^4	
	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid	Control	Gibberellic acid
8 days after spraying														
1	128 ± 21	129 ± 23	0·14 ± 0·02	0·14 ± 0·03	1·4 ± 0·2	1·4 ± 0·3	5·9	6·2	2·6	2·5	388 ± 53	398 ± 24	2·16	2·10
2	123 ± 7	115 ± 9	0·14 ± 0·01	0·12 ± 0·01	1·4 ± 0·1	1·4 ± 0·2	5·6	5·8	2·7	2·7	385 ± 25	354 ± 28	2·18	1·96
3	101 ± 14	94 ± 11	0·13 ± 0·01	0·14 ± 0·03	1·2 ± 0·1	1·4 ± 0·2	3·9	4·5	3·2	3·3	369 ± 43	256 ± 32	2·62	2·11
4	63 ± 3	59 ± 11	0·12 ± 0·01	0·14 ± 0·02	0·82 ± 0·2	1·1 ± 0·2	1·8	2·1	2·8	3·3	209 ± 37	145 ± 10	3·73	2·85
15 days after spraying														
2	106 ± 3	99 ± 5	0·098 ± 0·01	0·12 ± 0·01	0·94 ± 0·05	0·89 ± 0·06	4·4	5·0	2·6	2·7	—	—	2·40	1·85
3	100 ± 25	81 ± 29	0·12 ± 0·02	0·12 ± 0·02	0·91 ± 0·07	0·91 ± 0·1	3·6	4·2	2·9	2·7	288 ± 48	246 ± 20	2·77	1·94
4	72 ± 11	61 ± 14	0·12 ± 0·01	0·13 ± 0·01	0·93 ± 0·06	0·98 ± 0·1	2·3	4·0	3·2	3·1	262 ± 35	169 ± 32	2·49	1·55
5	80 ± 18	57 ± 11	0·15 ± 0·02	0·16 ± 0·02	1·2 ± 0·3	1·1 ± 0·1	2·3	3·0	2·9	2·8	185 ± 39	140 ± 15	3·46	1·90

It can be concluded from the above results that the appearance of chlorosis in material treated with gibberellic acid is not due to lower chlorophyll content per cell but is apparently an artefact arising from the effect of gibberellic acid on leaf expansion. This results in the treated material having leaves of greater surface area which are thinner than the controls and thus having a shorter optical path for absorption of light. Similar results were obtained in two successive seasons, the hot bright summer of 1959, and the cooler wet summer of 1960. Our results are in agreement with those of Wolf and Haber[4]. In their case with wheat seedlings an influence of nutritional factors on the chlorophyll content became apparent in older plants; this was not observed with the meteor pea plants grown in the field.

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Effect of Auxin on the Osmotic Value in Pea Stem Sections

The osmotic concentration in pea stem pieces was found to be increased by indole-3-acetic acid (IAA) when they were smeared with lanolin paste of IAA and kept at constant humidity[1]. Treatment with auxin solution was avoided in this case in order to exclude the possibilities of a secondary osmotic change due to water absorption and leaching of solutes from cells. In the experiment to be reported here, however, auxin solution was used in order to observe an osmotic change immediately following the auxin action.

Excised pieces, 10 mm. long, from etiolated stems of Alaska peas were weighed and floated on 10 and 200 mgm./litre solutions of IAA and on water at 25·5° C. Samples were withdrawn at intervals, blotted with filter paper, and the freezing point depression of tissue juice was determined to the nearest 0·01° C.[1], using a thermojunction. The freezing point of the juice was around − 0·56° C. before the tissue was introduced into the solutions. Changes of freezing point in the solutions are shown in Fig. 1.

The osmotic concentration at first increased, following exposure to IAA, and then decreased rapidly after 30 min. or so. In the water control, on the other hand, no temporary increase in osmotic concentration was observed. Experiments not presented here showed that phenylacetic acid and acetic acid in the molar concentration equivalent to 200 mgm./litre of IAA did not cause a rise in osmotic concentration as IAA did.

No significant difference in the rate of water absorption was found between the two auxin-levels in the first 30 min., even though the osmotic values at the two levels differed greatly. Water permeability may have been a limiting factor.

The drop in osmotic concentration may be ascribed to (1) dilution due to water absorption (which is calculable) and (2) leaching of solutes from cells to outer solution. The more rapid drop in the presence of IAA may probably be ascribed to an increased permeability[2–5].

It has been reported that the osmotic concentration is increased by IAA[7,8]. It is, however, worthy of note

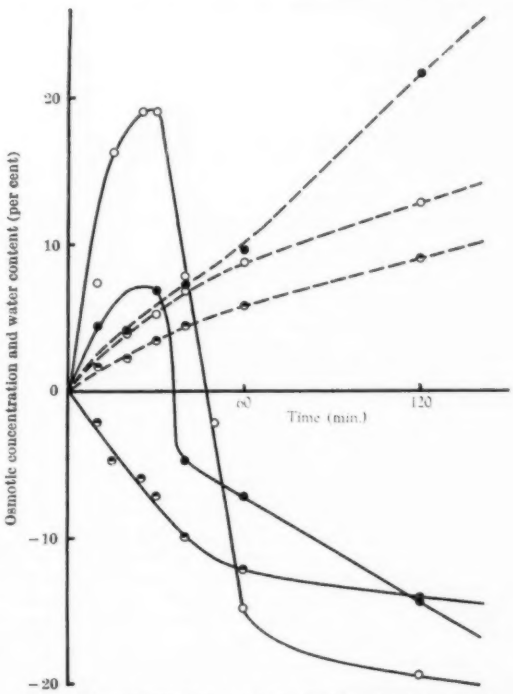

Fig. 1. Water absorption (– – –) and osmotic concentration (—) of pea stem sections treated with water (○), and 10 mgm. (●), and 200 mgm. (○), of indole-3-acetic acid per litre

that the osmotic effect appears in such a short time as shown here. In order to see how rapidly the cell wall extensibility is affected by auxin, longitudinal tension of 2·5 gm. was applied to stem pieces soaked for various periods in 200 mgm./litre solution of IAA. By this method[9,10], an increase in extensibility became observable in 15 min. of auxin action, but not in 10 min. Hence the osmotic change occurs earlier than the change in cell wall extensibility.

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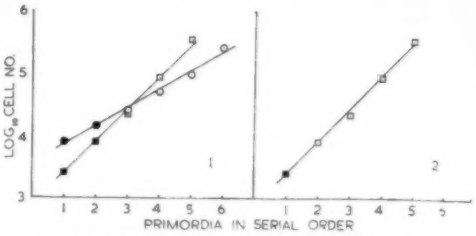
Determination of Cell Numbers in Leaf Primordia

Sunderland and Brown[1] have pointed out that there are two major problems involved in the determination of the number of cells in leaf primordia: (1) numerous macerating fluids[2] tend to disintegrate the relatively delicate cell wall system of leaf primordia and (2) owing to the minute size of leaf primordia and their relatively small number of cells, fragmentary losses are a serious source of error when counting by hæmocytometer slide after dilution.

A method is suggested here which mainly overcomes these difficulties and has been used successfully for the second to the sixth primordia of tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabaccum*) stem apices. It has greater ease and rapidity of counting than the squash method originally used by Sunderland and Brown[1] and can be directly integrated with values obtained by other techniques (for example, chromic acid) for larger leaflets[3].

Primordia were excised under a dissecting microscope using a small splinter from a safety razor blade gripped in a holder. Immediately after excision the primordia were placed in acetic acid/ethanol, 1 : 1 (v/v), for at least 10 min. at room temperature and then treated with *N* hydrochloric acid for 8–15 min. at 60° C. in an oven, depending on the size of the primordia[1]. The primordia were immersed in the liquid in wells constructed by placing on a slide up to three rings cut in lengths of 3 mm. from polyvinyl chloride tubing (10 and 15 mm. diameter) with a razor. During the treatments the slide rested on the rim of a watch glass, which stood in a Petri dish containing the same liquid as the slide in order to reduce evaporation of the liquid[1]. After the removal of each reagent by applying filter paper strips to the base of the outside of the well, the polyvinyl chloride rings were removed with a pair of forceps without disturbing the primordia. The primordia were then removed from the slide under the dissecting microscope, using a fine glass rod or a filter paper strip, and transferred to 10 × 0·6 cm. (approx.) specimen tubes containing a precisely known volume



Figs. 1 and 2. Tomato, ○, hæmocytometer; ●, squash; tobacco, □, hæmocytometer; ■, squash

—between 0·3 ml. and 1·0 ml.—of 0·1 *M* ethylenediaminetetraacetic acid in 5 per cent (v/v) aqueous butanol mixture at *p*H 8 (ref. 4) using ammonia solution to adjust the *p*H. The tubes were tightly stoppered and the primordia in the ethylenediamine tetraacetic acid/butanol mixture were incubated overnight at 30° C. The primordia were then macerated gently with a small syringe (1·0 ml.) to which a finely drawn out piece of glass capillary tubing was attached with surgical polythene tubing. After maceration, 0·1 ml. of 1 per cent (w/v) malachite green in water was added to each tube to produce a minimum of 0·4 ml. for the smallest primordia; for larger primordia the volume was made up to 1 or 2 ml. and the volume of the stain increased proportionally. The suspension was again drawn a few times through the syringe to ensure uniform distribution of cells and 0·2 ml. was transferred to a hæmocytometer slide (Fuchs–Rosenthal; depth, 0·2 mm. area, 1/16 mm.^{2}) and counted. Duplicate estimations on separate aliquots were made for each primordium.

Preliminary results obtained with this method for the second or third and older primordia of tomato and tobacco plants aged 8 weeks in November and grown in water culture are shown in Figs. 1 and 2, in relation to those obtained for the first and second primordia by the squash method of Sunderland and Brown[1]. For the particular purpose of these comparisons plants about 8 weeks old were selected when the youngest primordium visible on the apex (numbered 1) had attained what was judged to be almost the maximum size before the next was due to appear. In Fig. 2, all primordia except the first were measured by the hæmocytometer method.

The numbers of cells counted for each aliquot of 0·2 ml. ranged between 60 and 600 according to primordial position and species. The standard error of the means of 18 pairs of counts was ± 3·7 and the mean difference between pairs did not differ significantly from zero. These counts were about 0·5 or 1 per cent of the total cell number for the smaller primordia (Nos. 3 or 2) and 0·1 per cent of the total for the sixth primordium. The extension of counts to more mature leaves using chromic acid maceration[3] showed a linear relationship for log cell number up to about the eighth or ninth leaf in both species.

We thank G. M. Clarke for statistical treatment and the British Council for an Overseas Scholarship to one of us (L. A. W.).

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Potential Productivity of Marrow Stem and Thousand-headed Kale

SEED of marrow stem kale was sown in August and the plants transplanted to permanent quarters in October. Approximately half of the plants flowered in the following spring. The non-flowering plants continued their vegetative growth and were sampled by the 'perfect plant method' in the following October. Dry-matter production (excluding roots) was equivalent to 9·7 (from an August 4 sowing) and 7·6 (from an August 19 sowing) tons per acre. If we neglect the weight of the plants at transplanting time this represents a complete year's production of dry matter. Plants from later August and September sowings, transplanted in April, produced by October 8·6 and 6·2 tons of dry matter (excluding roots) per acre. Comparable production figures for thousand-headed kale from these later sowings were 7·7 and 6·2 tons. These figures represent a production of dry-matter two or three times as great as that obtained when the kales are sown in the traditional manner in April, and much in excess of that obtained with other agricultural crops. They may be compared with the annual dry matter production of 7·6 for the evergreen *Pinus*[1], 3·2 for birch[2], and 1·6–5·6 tons per acre for natural herbaceous vegetation[3].

The high productivity of the kales undoubtedly results from their early development of a full leaf cover, the autumn-transplanted marrow stem kale having a mean leaf area index of 0·4 by early May and one of approximately 2·0 by the end of that month, which compares with a leaf-area index of 0·06 at that date for a commercial crop sown in April as is customary, and which yielded 3·3 tons of dry matter per acre.

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Cytochrome c Oxidase, the Terminal Oxidase of *Iris pseudacorus* L.

WHILE it is generally agreed that cytochrome *c* oxidase is the terminal oxidase of embryonic plant tissues, some workers doubt if it has this role in older plant tissues as well[1]. It is of interest that a mature tissue such as the rhizome of *Iris pseudacorus* L., obtained from a Lancashire marsh, should contain a cytochrome system which might reasonably be claimed to account for the oxygen uptake of the tissue. The rhizome tissue used was cut from within the endodermis of the current year's growth, 1·0 cm. or farther from the meristematic region. The cells were typical parenchymatous cells except that the tissue also contained some vascular cells.

The presence of a cytochrome system was apparent from the absorption spectra of slices of reduced tissue when examined with a direct-vision spectroscope. The positions of the maxima of the absorption bands in the visible region corresponded approximately with the $(a + a_3)_a$, b_a and c_a cytochrome bands of a reduced yeast suspension with which they were compared, except that the b_a and c_a bands of the *Iris* tissue were not distinctly separated.

Cores of tissue were used for respiration experiments. These cores 1 cm. diameter and 2·5 cm. long were split lengthways, rinsed and used immediately. The uptake of oxygen of the 'half-cores' was measured in Warburg manometers at 10° in a damp atmosphere of 95 per cent carbon monoxide/5 per cent oxygen or 95 per cent nitrogen/5 per cent oxygen both in the light (250-W. mercury vapour lamp) and in the dark. A suspension of baker's yeast in 0·1 *M* phosphate buffer, *p*H 6·0, was also used as a comparison and the dark carbon monoxide inhibition was 60 per cent in both cases and fully reversed in the light (Table 1).

Table 1. THE EFFECT OF CARBON MONOXIDE/OXYGEN AND NITROGEN/OXYGEN GAS MIXTURES ON THE RESPIRATION OF *Iris* RHIZOME AND BAKER'S YEAST

Organisms	Inhibitor	Per cent inhibition as compared with controls in an atmosphere of 95 per cent nitrogen/5 per cent oxygen
Rhizome tissue	95 per cent carbon monoxide/5 per cent oxygen in dark	60
	95 per cent carbon monoxide/5 per cent oxygen in light	0
Baker's yeast	95 per cent carbon monoxide/5 per cent oxygen in dark	60
	95 per cent carbon monoxide/5 per cent oxygen in light	0

It is generally accepted that cytochrome *c* oxidase is the terminal oxidase of baker's yeast and the fact that a similar light-reversed carbon monoxide inhibition of 60 per cent was obtained with *Iris* is evidence that a similar state of affairs exists in this tissue. These results would support the widely held view that cytochrome *c* oxidase forms the main terminal oxidase even in mature plant tissues[2].

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GERONTOLOGY

Rate of Senescence

WE owe to Minot[1] a startling paradox—the view that the rate of senescence is most rapid at the beginning of life and slowest at the end: to quote the fourth of the laws by which he summarized his conclusions, "Senescence is at its maximum in the very young stages and the rate of senescence diminishes with age". The justification for his view was that the intrinsic or specific growth-rate of the tissues decreases most rapidly at the outset and progressively more slowly afterwards, following a roughly exponential die-away curve (Fig. 1,*A*). It seemed a reasonable assumption that the senescent state is related to the loss of the power of growth and so the paradox has been widely accepted, notwithstanding the intuitive conviction that the process of senescence is not significant until after the prime of life. The postulated initial, rapid stages of senescence have to be shrugged off as 'insensible'.

However, there are properties relevant to senescence which can be measured and which are not found to increase most rapidly at the outset: the specific death-rate, for example, is minimal not at the outset but in late childhood; it then begins to increase slowly, with progressive acceleration[2], and not most rapidly at first. It is therefore worth

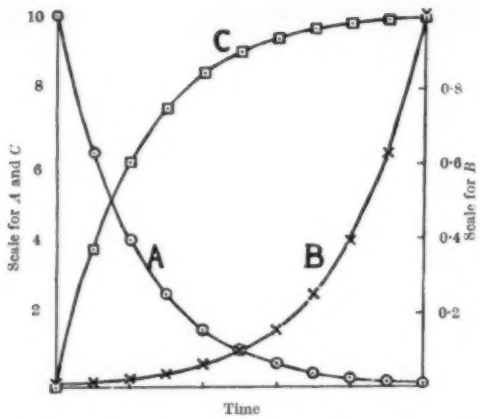

Fig. 1. Graphs against time of (*A*) specific growth-rate in arbitrary units, (*B*) its reciprocal, (*C*) its negative (deficit below its maximal value)

looking into the basis for Minot's view on the rate of senescence. Most people probably believe that his relation implies that the state of senescence is proportional to the reciprocal of the specific growth-rate and accept it on this understanding. However, if the reciprocal of the values of the specific growth-rate in Fig. 1.*A* are plotted on a suitable scale (Fig. 1,*B*) the curve is a climbing exponential, convex and not concave to the time axis, the rate increasing most slowly at first and progressively more rapidly afterwards ; this is in keeping with the specific death-rate and other criteria, as well as with intuitive judgments. Minot's relation really assumes that the state of senescence is proportional to the negative of the specific growth-rate (Fig. 1,*C*) or more precisely to its deficit below an initial maximal value; that is, to $(k-x)$ rather than to k/x. This has much less appeal on the score of biological rationale, and so there is neither a very strong argument, nor much evidence, in favour of his relation, with its grotesque paradox. Its rejection should greatly clarify the situation and stimulate further work.

Even the reciprocal relationship between senescence and growth-rate is only acceptable with a certain stipulation. In man increase in height and weight ceases around the age of thirty. At this early age, therefore, when he is manifestly in his prime, the reciprocal of the specific growth-rate tends to infinity. Soon after this time actual shrinkage begins, and the specific growth-rate becomes negative. Consequently, its reciprocal now swings sharply to $-\infty$ and then climbs to finite but permanently negative values. To convert this into biological sense it is necessary to assume that the relevant growth-rate is not mere increase in size but that of growth in the broadest sense, including maintenance and repair—perhaps in fact anabolism *in toto*[3]. Growth in this sense remains positive throughout life, though eventually unable to keep pace with catabolism. It does not imply an infinite rate of senescence at any stage, therefore. With this modification, moreover, senescence is not maximal at the age of thirty : there is no discontinuity at that age, anabolism continuing a smooth decline.

There is a further point which deserves attention. The rate of shrinkage in man, as shown, for example, by Quetelet's data[4], does not increase to the end but is maximal between sixty and seventy years of age, and slower in the very old. Consequently the specific growth-rate increases again in old age. There is direct evidence of this in the rate of cell division in some tissues[5]. Other properties also reverse their trend in later life, and if this is true of senescence, also, it may provide some slight justification for the popular jest about second childhood[5].

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MICROBIOLOGY

Physical Conditions as Important Factors for the Regeneration of Naked Yeast Protoplasts

In our previous papers[1] we have discussed the growth and regeneration of naked yeast protoplasts of *Saccharomyces cerevisiae* obtained by means of cell wall autolysis. In the former experiments the growth and regeneration took place on solid synthetic media. The frequency of regeneration was low on these media; while nearly all the protoplasts were capable of growth, in fact only about 1 per cent regenerated into new yeast cells (in a complicated manner formerly described).

Studying the factors influencing regeneration, we have found that practically all the protoplasts are capable of regeneration under suitable conditions. We were successful in finding conditions under which the protoplasts regeneration is nearly 100 per cent.

Protoplasts do not regenerate in liquid media at all. The frequency of regenerations is very low on the surface of solid agar media and the chemical composition of the media does not influence considerably the frequency of regenerations. But nearly 100 per cent regeneration can be achieved 24 hr. after inoculation by cultivation of protoplasts inside solid media containing gelatin (see Table 1). (On the surface of agar media regeneration does not take place before 5 days.)

It is seen from Table 1 that the concentration of gelatin is important. Regeneration is not accomplished in gelatin with concentration less than 15 per

Table 1

Medium	Mode of cultivation	Concentration of gelatin (per cent)						
		0	5	10	15	20	30	40
Synthetic	On the surface of gelatin	0	0	0	0*	0*	0*	0*
	Inside gelatin	0	0	0	Reg. + −	Reg.	Reg.	Reg.
Synthetic + 30 per cent of gelatin hydrolysate	On the surface of gelatin	0	0	0	0*	0*	0*	0*
	Inside gelatin	0	0	0	Reg. + −	Reg.	Reg.	Reg.

* Only the protoplasts which have penetrated into gelatin regenerate.
0, No regeneration; *Reg.* + −, sporadic regeneration; Reg., nearly all the protoplasts regenerate.

cent (at a temperature of 22° C.). Protoplasts growing on the surface of gelatin of any concentration do not regenerate, but protoplasts have proteolytic activity and can penetrate into the gel, where they can regenerate. The contact of a protoplast with gelatin gel is not sufficient for regeneration; protoplasts have to be surrounded by the gel. Further, we can see from Table 1 that regeneration does not take place in media containing 30 per cent of hydrolysate of the gelatin, unless a gel of unhydrolysed gelatin of proper concentration is used. Thus, regeneration is not influenced by the chemical composition of gelatin. Regeneration is not, likewise, accomplished in liquid media with albumin of different concentrations.

It may be concluded, therefore, that nearly all the yeast protoplasts have not only the ability to grow but also capacity for regeneration, and that physical conditions, that is, surrounding of protoplasts by the gel of certain consistency, are important for regeneration. The explanation of the mechanism of this process will be considered later.

These results, as well as the micro-cinematographic registration of the whole process, seem to disprove the opinion of Eddy and Williamson[2] that in naked yeast protoplasts there is not an autoregeneration, but a formation of cells by budding of spores accidentally present in the suspension of protoplasts.

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VIROLOGY

Transmission of Clover Phyllody Virus by *Aphrodes bicinctus* (Schrank) in North America

Phyllody, a disease of clovers, occurs in eastern North America and appears to be similar or identical to the disease present in England, continental Europe and the U.S.S.R. In England, Frazier and Posnette[1] first demonstrated that a virus, transmitted by certain species of leafhoppers, is responsible for causing phyllody in clover, and that the same virus, transmitted by dodder, is responsible for causing green-petal of strawberry. Leafhopper vectors of this virus recorded to date include *Euscelis lineolatus* Brullé[1], *Macrosteles viridigriseus* (Edwards)[1], *Aphrodes bicinctus (Schrank)*[2,3], *Aphrodes albifrons* L.[4] and *Macrosteles cristatus* (Ribaut)[4]. The work reported here was undertaken to determine if the disease occurring in clover in North America is also caused by a leafhopper-borne virus.

A. bicinctus collected from both strawberry and clover plants was shown to be capable of transmitting the clover phyllody virus from diseased to healthy ladino clover *Trifolium repens* L. Nymphs collected from strawberries at Ottawa, located 150 miles from the western limit of the disease, were first caged on healthy ladino clover for approximately three weeks, after which they were allowed to feed on infected ladino clover for 18 days. The insects were then transferred at intervals to healthy ladino plants. The leafhoppers were shown to be non-viruliferous when captured but to be capable of transmitting the virus to healthy plants after feeding on diseased plants. All six healthy plants later showed signs of virus infection (chlorosis, bronzing and stunting) and five out of the six plants developed phyllody symptoms. The remaining plant failed to flower.

Three adults of *A. bicinctus*, collected at Lavaltrie, Quebec, from a field of clover where phyllody was naturally present, were allowed to feed on disease-free ladino clover for one week to determine if they carried the virus. They were then allowed to feed for nine days on ladino clover which was infected with phyllody virus, after which they were transferred to virus-free ladino clover plants at irregular intervals until the last insect died. The leafhoppers were not viruliferous when captured but became infective after feeding on diseased plants. They transmitted the virus to each of four healthy test plants on which they were serially transferred. These plants later displayed typical leaf symptoms. Three out of the four clover plants developed phyllody.

These experiments demonstrate that *A. bicinctus* is capable of transmitting the virus that causes clover phyllody in North America. A search for additional vectors of the virus in eastern Canada is in progress.

This is believed to be the first report of transmission of the virus causing clover phyllody by a leafhopper in North America.

Grateful acknowledgment is extended to Dr. W. R. Richards, Entomology Research Institute, Ottawa, for identification of *Aphrodes bicinctus* (Schrank).

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Movement of Tobacco Mosaic Virus through Epidermis of *Nicotiana glutinosa* Leaves

In detached primary cowpea leaves, cucumber mosaic virus requires about 2 hr. at 28° C. to pass through the epidermis to the palisade and to cause local lesions[1]. Also, other reports indicate that it takes a certain time for tobacco necrosis virus to move from the infection site on leaves of French bean (*Phaseolus vulgaris* L. var. Prince) into the palisade layer and that the rate of such movement is greatly influenced by the temperature[2]. Uppal reported that tobacco mosaic virus moved from the upper epidermis of *N. sylvestris* to the lower epidermis at a rate of 7–8 μ/hr. He was able to detect the virus in the palisade layer about 4 hr. after inoculation of the upper epidermis[3].

A number of experiments was performed in order to investigate the movement of tobacco mosaic virus through the upper leaf epidermis of *N. glutinosa*. Detached leaves of medium size were lightly dusted with 600-mesh 'Carborundum' and were brush-inoculated[4–6]. The brush was softened before use by immersion in water for a few minutes. The leaves were thoroughly washed with tap-water immediately after inoculation and kept on wet paper towels at room temperature (22–24° C.). By means of a very fine pointed forceps, strips of the upper epidermis

were taken 3, 3½, 20 and 21 hr. after inoculation. The experiment was repeated several times. No local lesions appeared on the stripped area even 48 hr. after stripping, whereas about 150 local lesions appeared 23–26 hr. after inoculation on the rest of the leaf. On the other hand, bio-assays of areas devoid of epidermis revealed that virus was always present even after only 3 hr. In another experiment, portions of the epidermis of detached leaves were stripped off and 20 hr. later the stripped areas were then inoculated in the usual manner. No local lesions appeared on the stripped areas.

Stripping of the upper epidermis is very difficult, presumably because the epidermal layer is tightly connected to the underlying palisade cells. The strips were examined under the low magnification of a compound microscope and appeared to consist of the epidermal cells plus a thin portion of the palisade layer. The palisade layer was therefore rather heavily damaged in the stripping process.

It appears that in detached *N. glutinosa* leaves tobacco mosaic virus penetrates through epidermal strips into the palisade in less than 3 hr., but that for some reason under my conditions, the presence of epidermis is required for necrotic lesion formation.

This work was done while I was a research assistant at the University of California and was supported in part by a grant No. *E*–3415 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

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Utilization of Sheep Red Blood Cells in the Hæmadsorption Test for Myxoviruses

The hæmadsorption test[1,2] has been found to be a simple and useful means of detecting foci of infection by myxoviruses in tissue culture monolayers. The same response may be observed in cell cultures whether these viruses are non-cytopathic[3] or cytopathic. Briefly, the technique consists of decanting the growth fluid and covering the monolayer with a 0·4 per cent saline suspension of guinea pig or chicken red blood cells and allowing hæmadsorption to proceed for 10 min. at 4° C. After two saline washes, the areas of hæmadsorption are examined under low magnification.

Although divergent results have been reported on the ability of different myxoviruses to agglutinate sheep red blood cells[4–7] it was found in this laboratory that commercial sheep red blood cells (Brown Laboratory, P.O. Box 424, Topeka, Kansas) may be used effectively in the performance of the hæmadsorption test. Hæmadsorption was observed in a continuous cell line isolated from human kerato-acanthoma with the following egg-adapted strains of myxoviruses: influenza *A* (*PR*8, and *FM*1, *A*2) (Japan 305), *B* (Lee and *B*54), and mumps virus. Controls consisted of normal uninoculated cells and infected cells to which a 1 : 10 dilution of specific rabbit virus antiserum or normal rabbit serum was added 10 min. prior to the addition of sheep red blood cells. In all cases the controls showed no hæmadsorption. It was also possible to show hæmadsorption to sheep red blood cells with an influenza *C* strain in a continuous line of cells isolated from rabbit heart.

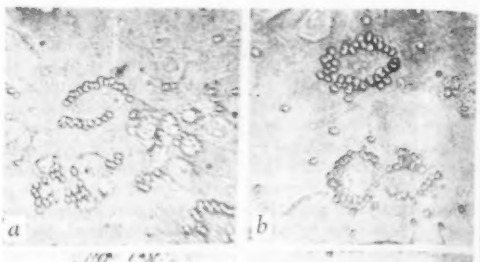

Fig. 1. Utilization of sheep red blood cells in the hæmadsorption test for myxoviruses

Fig. 1 shows hæmadsorption 'rosettes' seen with myxoviruses and the kerato-acanthoma cell line. Very little difference is seen between the response in the case of mumps virus and two of the influenza *A* strains (*PR*8, and *FM*1) tested (Figs. 1*a* and *b*). In the case of the *A*2 strain (Fig. 1*c*), however, the 'rosettes' often take on a much heavier appearance. The reason for this occurrence is not known, but it may be related to the amount of 'free' virus on the cell sheet. Distinct 'rosettes' are not seen as often with influenza *B* viruses (Lee, and *B*54) because of the toxicity and rapid destruction of the cells *per se* by these viruses (Fig. 1*d*).

The advantage of using sheep red blood cells for the hæmadsorption test is that they are readily accessible commercially and may be used for periods up to 3 weeks or longer when stored at 4° C.

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TIMBER TECHNOLOGY

Effect of Temperature on the Strain Recovery in Wood

Until recently, strains occurring beyond the limits of plastic creep in wood were, because of the similarity to creep in crystalline solids, identified with micellar creep in cellulose and considered as plastic strains[1]. Investigations carried out by Ivanov[2], in which swollen wood was subjected to cyclic loading and unloading with compression stresses above the limit of plastic creep along the fibre direction, showed that after each cycle of loading and unloading, both elastic and residual strains are increasing. Moreover, Ivanov found that strains occurring during compression beyond the limits of plastic creep, when wood is compressed along the direction of fibre length until the moment of showing symptoms of failure, were not connected with microscopic displacement of wood structure components. On the other hand, strains occurring beyond the limits of plastic creep when wood is compressed across the fibre direction are, to some extent, related to the deformations of cell cross-section shapes[3].

On the basis of present available research results, strains occurring in wood can be divided into three categories: elastic strains, elastic lagged strains, and plastic strains.

Investigations on the stress relaxation in wood showed that stresses are undergoing relaxation with the passage of time regardless of whether they are higher or lower than those occurring at plastic creep limit[4,5].

Moisture content and temperature of wood are exerting a decisive effect on the relaxation of stresses in such a way that the increase of moisture content and temperature is accelerating stress relaxation[6].

We have now determined the effect of temperature on the strain recovery in wood after compression across the fibre direction. The detailed results of these experiments are to be published in the near future. Experiments which are the subject of this report were conducted on green, maximum swollen beech-wood (*Fagus sylvatica* L.). Samples of dimensions 20 × 20 × 30 mm. were subjected to compression with a thrust amounting to 120 per cent of ultimate strength (σ_D). For ultimate strength, the stresses corresponding to the limit of plastic creep were assumed.

Experiments consisted of the following independent treatments: (1) loading of wood at 20° C. followed by heating it to 100° C. after a lapse of 24 hr. from the moment of unloading; (2) loading of wood at 20° C. followed by heating it to 40, 60, 80 and 100° C. immediately after unloading; (3) loading of wood at 20, 40, 60, 80 and 95° C. and observing the strain recovery at the same temperatures.

Because the wood was wet and in a state of maximum swelling, water used as a heating medium was raised to the predetermined temperature in order to eliminate the possible effect of wood moisture-content changes on the results of observations. In the evaluation of test results, the correction resulting from anomalies of the coefficient of linear temperature strains in moist wood was introduced.

Maximum swollen wood, when heated in water, is subject to elongation in a tangential direction and contraction in radial and longitudinal directions[7–9].

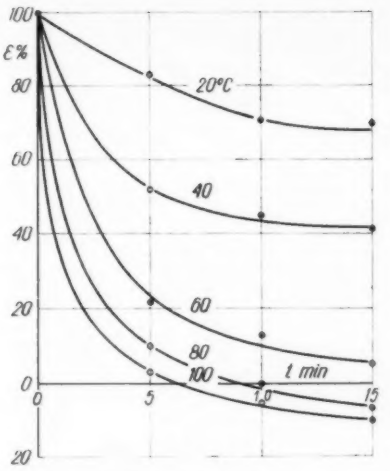

Fig. 1. Effect of temperature on the strain recovery in beech-wood compressed across the fibre direction

Experiments in the first treatment showed that wood, like other substances with large molecules of chain structure[10], is also subject to the thermo-elastic strain recovery effect. This phenomenon appeared also when the temperature of wood, previously steamed for 6 hr., was increased from 20° C. to 100° C.

The effect of temperature on the strain recovery, expressed in percentage of strain, and observed immediately after unloading, is illustrated in Fig. 1. It is evident that the rate of strain recovery increases with the rise of temperature. Also it may be noted that at 80° C. and 100° C., wood sample dimensions in the direction of compression exceed initial dimensions in that direction.

From Fig. 2 it may be seen that wood previously heated to various temperatures and loaded with the same compressing force (120 per cent σ_D) recovers its initial dimensions almost completely when heated during loading, and after unloading maintains these dimensions regardless of the magnitude of strain which occurs during loading.

From the results obtained it is evident that when maximum swollen wood is heated it acquires a high degree of elasticity. There is a probability that the total strain occurring at some moment after unloading the wood sample, and regarded commonly up to now as a plastic strain (durable, non-recoverable), is only apparently plastic, being the sum of thermo-elastic and real elastic strains. The magnitude of real elastic strain is intrinsically dependent on wood temperature after unloading of the stress. In the case of compressing beech-wood across the fibre direction the strain, which was regarded up to now as a plastic one, proved to be almost entirely recoverable and merely temporary fixed thermo-elastic strain.

The above-mentioned phenomena occurring in wood can be explained partly by a characteristic property of cellulose, the main component of wood structure, and that is its aptitude to change into a highly elastic state. This transition results from the increase of elasticity of molecular chains (mainly by the increase of oscillation amplitude) with the rise of temperature, as is the case with other polymers[11].

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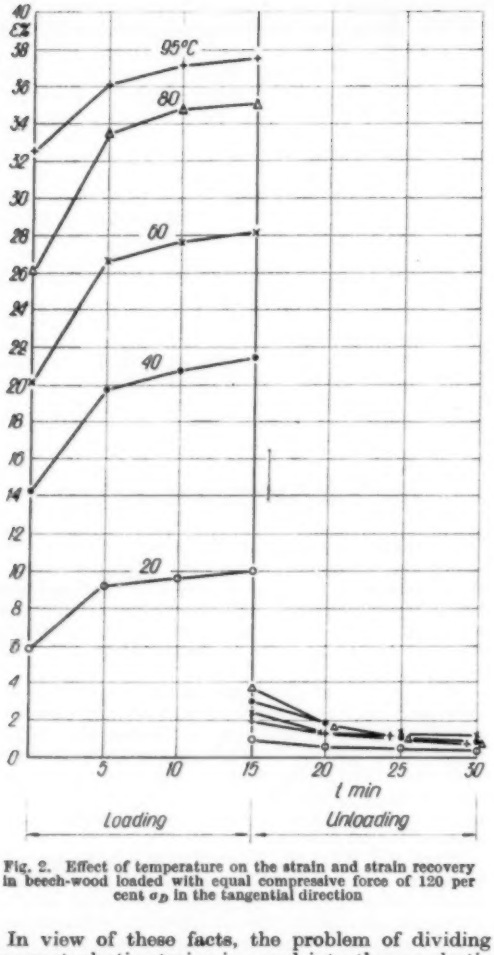

Fig. 2. Effect of temperature on the strain and strain recovery in beech-wood loaded with equal compressive force of 120 per cent σ_D in the tangential direction

In view of these facts, the problem of dividing apparent plastic strains in wood into thermo-elastic and real plastic strains is of great importance.

On the basis of these experiments we propose the partition of strain recovery in wood according to the scheme presented in Fig. 3. Only those strains

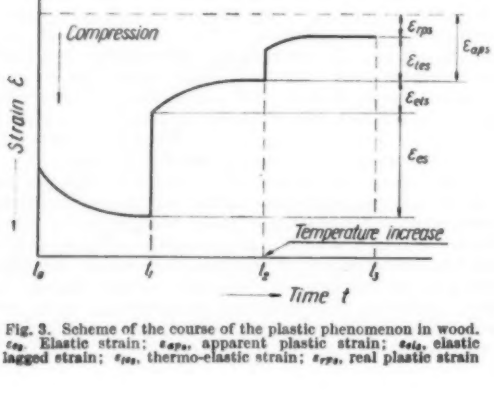

Fig. 3. Scheme of the course of the plastic phenomenon in wood. ε_{ap}, Elastic strain; ε_{aps}, apparent plastic strain; ε_{els}, elastic lagged strain; ε_{tes}, thermo-elastic strain; ε_{rps}, real plastic strain

which remain after the first thermoplastic effect can be considered as the real plastic strains (ε_{rps}). It was experimentally corroborated that further increase of temperature, for example in time t_3 (Fig. 3), results in very little, if any, increase of strain recovery. In order to determine the value of real plastic strain caused by compression of moist wood across the fibre direction, it is sufficient, it seems, to separate the thermoplastic strain (ε_{tes}) from apparent plastic strain (ε_{aps}) by accelerating strain recovery by raising the temperature of the wood.

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Poznań.

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MISCELLANEOUS

Philosophical Transactions of the Royal Society

In connexion with Dr. Wightman's article in *Nature* of Oct. 7, p. 23, on the relationship between the Royal Society and the *Philosophical Transactions* in the seventeenth and early eighteenth centuries and his emphasis on the unofficial character of the *Transactions*, it may be interesting to note that the abridgement of these up to the year 1700, prepared by John Lowthorp, appears to have been officially sponsored by the Society. The fly-leaf of the third edition, printed in 1722 after the publication in the preceding year of a further abridgement of the *Transactions* for the years 1701–20 by Benjamin Motte, carried the following advertisement :

"May 5, 1703. At a meeting of the Royal Society, Sir John Hoskyns, V.P. in the chair, Mr. Lowthrop Presented a Proposal for printing an Abridgement of the Philosophical Transactions. This Design was Approved by the Society and He was Desir'd to proceed therein."

This is followed by the subscription :

"May 12. 1705
Impimatur
Is. Newton, R.S.Pr."

(Lowthorp's name is so misspelt and the 'r' omitted from the word 'imprimatur' in my copy.)

In contrast, the abridgement by Motte carries only a commendatory notice, dated July 26, 1720, signed "Edm. Halley, Secr. Reg. Soc.".

O. M. Lidwell

Central Public Health Laboratory,
Colindale Avenue,
London, N.W.9.

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 13

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. F. A. E. Pirani and Dr. G. F. Elliott: "Ballistic Missiles and Nuclear Weapons—Nuclear Weapons"*. (Fifth of seven lectures in War Studies. Further lectures on November 21 and 27.)

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.45 p.m.—Mr. R. W. Leslie: "The Work of the B.B.C., with particular reference to Transmitters".

Monday, November 13—Saturday, November 18

ENGINEERING MATERIALS AND DESIGN EXHIBITION (at Earls Court, London).*

Tuesday, November 14

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London N.W.1), at 4 p.m.—Dr. J. T. Stamp: "Scrapie—a Transmissible Disease of Sheep".*

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. N. Parkman: "Tracking in Insulation".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. B. J. Mason: "The Physics of Clouds, Rain and Lightning" (Inaugural Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. V. R. Born: "Blood Platelets".* (Ninth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. H. O. Schild: "Adventures with Histamine"* (Inaugural Lecture).

UNIVERSITY OF LONDON (at the Institute of Archæology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Mr. Emmanuel Anati (Hebrew University, Jerusalem): "The Rock Engravings of the Camonica Valley, North Italy".*

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. Julius Mackerle: "Automobile Air-Cooled Engines".

INSTITUTION OF AGRICULTURAL ENGINEERS (at 6 Queen Square, London, W.C.1), at 6.45 p.m.—Mr. H. J. Nation: "The Use of Hydraulics in Agricultural Machinery and Farm Tractors".

Wednesday, November 15

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Prof. N. H. Martin: "Chemical Pathology of γ-Globulin".*

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Scientific Papers.

INSTITUTE OF PETROLEUM (joint meeting with ASLIB, at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. R. Snel: "The Technologist and His Information Facilities".

BRITISH INSTITUTION OF RADIO ENGINEERS, RADAR GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. C. M. Cade: "Infra-Red Applications in Navigation".

INSTITUTION OF MECHANICAL ENGINEERS, NUCLEAR ENERGY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Influence of Gas-Inlet Temperature on Magnox Reactors".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. Riccardo Morandi: "Engineering and Architecture".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Controversial evening to ventilate outstanding points of doubt and dispute in corrosion science.

Thursday, November 16

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. H. C. Darby: "National Parks in England and Wales".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Special General Meeting. Prof. F. J. Fenner, F.R.S.: "Interactions between Poxviruses" (the Leeuwenhoek Lecture).

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting. Prof. H. A. Heilbronn: "Old Theorems and New Methods in Class-field Theory" (Presidential Address).

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. K. K. Schwarz: "The Design and Performance of High- and Low-Voltage Terminal Boxes".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. M. Daniel: "The Pituitary Gland and its Blood Supply".* (Tenth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. R. S. Millard: "Road Building in the Tropics".

INSTITUTE OF WOOD SCIENCE (at Carpenters Hall, Throgmorton Avenue, London, E.C.2), at 7 p.m.—Mr. G. B. Crow: "The Economics of Timber Engineering".

INSTITUTION OF ELECTRONICS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 7 p.m.—Mr. I. Aleksander and Mr. M. R. McCann: "Developments in Tunnel Diode Technology".

SOCIETY OF INSTRUMENT TECHNOLOGY, DATA PROCESSING SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. D. E. Cartwright, Mr. M. J. Tucker and Miss D. B. Catton: "Digital Techniques for the Study of Sea Waves, Ship Motion and Similar Processes".

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Prof. G. B. Kistiakowsky: "Some Reactions of Free Radicals" (Centenary Lecture).

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 7.30 p.m.—Laboratory Meeting. Chairman: Sir George McRobert.

Friday, November 17

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. F. D. K. Liddell: "Coalminers' Morbidity".

INSTITUTE OF NAVIGATION (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Captain William L. Polhemus, U.S.A.F.: "The Navigation and Guidance of Supersonic Aircraft".

UNIVERSITY OF LONDON DEPARTMENT OF EXTRA-MURAL STUDIES, in association with the BRITISH INSTITUTE OF RECORDED SOUND (at the British Institute of Recorded Sound, 38 Russell Square, London, W.C.1), at 7.30 p.m.—Mr. Peter Ford: "The Technique of Sound Recording—a Historical Survey". (Further lectures on November 24, December 1, 8 and 15.)

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. W. A. Mair: "Air Flow and Wings".

Friday, November 17—Saturday, November 18

BRITISH SOCIETY FOR IMMUNOLOGY (at the Wellcome Building, 183 Euston Road, London, N.W.1)—Autumn Meeting.

Friday, November 17

At 10 a.m.—Symposium on "Tissue Antigens". Chairman: Dr. L. E. Glynn.

INSTITUTE OF PHYSICS and the PHYSICAL SOCIETY, X-RAY ANALYSIS GROUP (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1)—Conference on "Imperfections in Crystals".

Saturday, November 18

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss Violet Alford: "Basque Traditions and Customs".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER or LECTURER (with special interests in fine structure or physiology) IN BOTANY—The Registrar, University College of Wales, Aberystwyth (November 18).

ASSISTANT LECTURER or LECTURER (graduate in veterinary science and/or agriculture) IN ANIMAL HUSBANDRY—The Registrar, The University, Liverpool (November 18).

BIOCHEMIST (Basic grade), for work on steroids—Clerk to the Governors, St. Bartholomew's Hospital, London, E.C.1 (November 18).

RESEARCH ASSISTANT IN STATISTICS—The Registrar, The University, Sheffield (November 18).

ORGANIC CHEMISTS, for posts in pure research—The Director, Brewing Industry Research Foundation, Nutfield, Surrey (November 20).

S.E.A.T.O. PROFESSOR OF ORGANIC CHEMISTRY at the University of Medical Sciences, Bangkok—The Director, Recruitment Department, British Council, 65 Davies Street, London, W.1, quoting Ref. N.12T (61) (November 20).

SENIOR RESEARCH ASSISTANT (post-doctoral standard, qualified in inorganic chemistry), to take part in an investigation of the crystal chemistry of the oxides and hydroxides of the transition metals—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (November 20).

ASSISTANTS (2) IN THE DEPARTMENT OF POTATO GENETICS, for general assistance in the conduct of experiments in laboratory, glasshouse and field—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (November 27).

ASSISTANT LECTURER (suitably qualified in pharmaceutical or organic chemistry) IN THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY—The Clerk to the Council, School of Pharmacy (University of London), 29–39 Brunswick Square, London, W.C.1 (November 30).

XUM

LECTURERS or ASSISTANT LECTURERS IN PHYSICS AND MATHEMATICS at the Royal University of Malta—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

LECTURERS (permanent or temporary) IN THE DEPARTMENT OF PHYSICS, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

SENIOR LECTURER or LECTURER IN BACTERIOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

LECTURER/SENIOR LECTURER IN ANIMAL PRODUCTION at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 2).

LECTURER (preferably with a special interest in physiology) IN THE DEPARTMENT OF ZOOLOGY, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 4).

ASSOCIATE PROFESSORS (2) (of high academic and professional standing, with special interests in (*a*) materials and structures, or (*b*) machines), at the Royal Military College of Science, Shrivenham, to teach at degree level, and to undertake and lead research in their own fields—Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. S/5404/61 (December 5).

CHAIR OF VETERINARY ANATOMY at the Royal Veterinary College—The Registrar, University of London, Senate House, London, W.C.1 (December 5).

LECTURER (with an honours degree in science or equivalent qualifications, and preferably a special interest in stratigraphy and structural geology) IN GEOLOGY at the University of New South Wales, Australia—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (December 8).

SOIL FERTILITY OFFICER (honours graduate in science or agriculture, preferably with background experience in soil chemistry, and some years relevant postgraduate research experience), at the Regional Pastoral Laboratory, Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Deniliquin, New South Wales, Australia, to study the role of organic matter in soil fertility changes under grazed pastures, particularly as it affects nutrient availability—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 132/100 (December 9).

SENIOR LECTURER or LECTURER IN CHEMISTRY at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 10).

LECTURERS (2) IN WILD LIFE CONSERVATION IN THE DEPARTMENT OF ZOOLOGY, University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 12).

CHAIR OF BOTANY in Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 15).

LECTURER IN PATHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 15).

LECTURER IN PHYSICAL OR INORGANIC CHEMISTRY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 15).

READER (experienced in teaching and research in a branch of experimental botany) IN BOTANY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 16).

ENTOMOLOGIST, for the study of blood-sucking arthropods, as part of a comprehensive investigation being carried out at the Institute into arthropod-borne viruses—The Director, Queensland Institute of Medical Research, Herston Road, Herston, N.9, Queensland, Australia (December 31).

F. H. LOXTON POSTGRADUATE STUDENT or SCHOLAR (male graduate of any Australian or overseas university) IN THE FACULTY OF AGRICULTURE—The Registrar, University of Sydney, Sydney, N.S.W., Australia (December 31).

MYCOLOGIST (with a degree in science, preferably with honours, or equivalent, and training in mycology) for investigation of airborne fungi in relation to allergic diseases—The Director, Queensland Institute of Medical Research, Herston Road, Herston, N.9, Brisbane, Queensland, Australia (December 31).

ASSISTANT LIBRARIAN (Chartered Librarian, preferably with previous experience in a scientific or technical library)—Northern Polytechnic, Holloway Road, London, N.7.

BIOCHEMIST (with postgraduate experience and preferably a knowledge of bacteriological techniques), to assist in research projects on bacterial polysaccharides—The Director, Central Public Health Laboratory, Colindale Avenue, London, N.W.9.

BIOLOGIST or ENTOMOLOGIST (preferably with an interest in biochemistry), for research on pesticides at the Tropical Products Institute, London, W.C.1, under the auspices of the Colonial Pesticides Research Committee—The Research Department, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1.

BISCUIT SCIENTIST, to lead a team working on the application of science to all aspects of the biscuit baking industry—The Director of Research, Baking Industries Research Station, Chorleywood, Herts.

CHEMIST or BIOCHEMIST (honours graduate), for research in allergy (work suitable for a higher degree)—The Secretary, Department of Parasitology and Entomology, Liverpool School of Tropical Medicine, Liverpool 3.

DISTILLERS COMPANY RESEARCH STUDENT, for work leading to a higher degree on the gaseous oxidation of hydrocarbons—The Secretary, University College, Gower Street, London, W.C.1.

HOSPITAL PHYSICIST, Basic grade (with a degree in physics), for work which includes treatment planning in a well-equipped Radiotherapy Department and clinical applications of radioactive isotopes—The Personnel Officer, St. Thomas's Hospital, London, S.E.1.

READER (with good academic qualifications supported by substantial experience in industry and/or research) IN CIVIL ENGINEERING—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

RESEARCH FELLOWS (2) IN THE DEPARTMENT OF MECHANICAL ENGINEERING, to carry out full-time research in one of the following fields: vibration phenomena in machine tools; control systems; friction and lubrication, including the performance of bearings; forming and machining processes—The Registrar, The Royal College of Advanced Technology, Salford 5.

SCIENCE GRADUATE (with honours in biology), for cytogenetic studies on human endocrine lesions—Prof. Symington, Pathology Department, Royal Infirmary, Glasgow.

SCIENTIFIC OFFICER (Geophysicist) (with a good honours degree in geophysics, physics or engineering, or equivalent qualifications and at least two years post-qualification experience) with the Federal Government of Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. J3/1.

SCIENTIFIC OFFICER (honours graduate in chemistry or biochemistry with interests in the isolation and chemistry of proteins and in studies on enzymes) IN THE PHYSIOLOGY DEPARTMENT—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, near Reading, quoting Ref. 61/16.

SENIOR LECTURER (qualified engineer with personality and experience to introduce an engineering bias, and the ability to plan courses and organize equipment for new laboratories) IN ENGINEERING; a LECTURER (with academic and/or professional qualifications together with industrial or research experience of rubbers and flexible plastics) IN PHYSICAL TESTING OF POLYMERS; and an ASSISTANT LECTURER (graduate or holding H.N.C. in chemistry) IN POLYMER CHEMISTRY—National College of Rubber Technology, Holloway, London, N.7.

SENIOR LECTURER (qualified to teach general polymer technology to final Diploma in Technology level, and to assist in the development of postgraduate courses in polymer science and technology) IN POLYMER TECHNOLOGY IN THE DEPARTMENT OF CHEMICAL TECHNOLOGY—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

SENIOR LECTURER (with an honours degree or equivalent in chemistry or chemical engineering, and recent experience of design and/or operation of chemical plants) IN CHEMICAL TECHNOLOGY—The Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref. CST.61/38.

SENIOR SCIENTIFIC OFFICER or SCIENTIFIC OFFICER (with a first- or second-class honours degree in mathematics or equivalent qualifications) at the National Institute of Oceanography, Wormley, Surrey, for research into applications of numerical analysis and for preparation of programmes covering a wide range of problems in oceanography; the work includes spectral analysis, data processing, solution of differential equations, etc.—The Ministry of Labour, Technical and Scientific Register (K), 28 King Street, London, S.W.1, quoting A.425/1A.

TECHNICIAN (fully qualified man or woman), to take charge, under the Chief Technician, of teaching laboratories in the present Department of Zoology and to organize the teaching laboratories in the greatly enlarged department now being built—Head, Department of Zoology, The University, Hull.

TECHNICIAN (interested in applying biochemical techniques to virology); and a JUNIOR TECHNICIAN (preferably with G.C.E. ("A" level) in chemistry) IN THE BIOCHEMISTRY DEPARTMENT—The Secretary, Research Institute, Pirbright, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Empire Cotton Growing Corporation. Descriptive Brochure. Pp. 17. (London : Empire Cotton Growing Corporation, 1961.) [258

London and Home Counties Regional Advisory Council for Technological Education. Bulletin of Special Courses in Higher Technology, including Management Studies and Commerce, 1961–62. Part 1: Autumn Term. Pp. 128. (London : London and Home Counties Regional Advisory Council for Technological Education, 1961.) 3*s*. 6*d*. [258

Associated Lead Manufacturers Limited. Lead for Radiation Protection. Pp. 18. (London : Associates Lead Manufacturers Limited, 1961.) [258

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

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